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Control of Pale Western Cutworm in Wheat[1]

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The pale western cutworm, *Agrotis orthogonia* Morr., has been a serious pest of wheat and other grains in the Great Plains region of North America since 1911. Sole reliance to protect wheat has been placed on cultural methods, but whenever these failed crops heavily infested were doomed to destruction. Recent tests by Jacobson *et al.* (1952) and by Hoerner (1953) have given hope for controlling this pest with insecticides. An outbreak of the pale western cutworm in the southeast corner of Wyoming, an area of extensive winter wheat production, provided an opportunity to retest some of the insecticides already tried and to test endrin, an untried material for pale western cutworm control.

METHODS.—Part of a heavily infested field was divided into three blocks of 13 plots each. Plots measured 20×100 feet. Ten different treatments and three checks were assigned at random to each block. Treatments consisted of 4 concentrations of endrin, 0.1, 0.2, 0.3, and 0.4 lb. per acre; 2 concentrations of dieldrin, 6 oz. and 0.5 lb. per acre; 2 concentrations of TDE, 1 and 2 lb. per acre; 1 concentration of heptachlor, 0.5 lb. per acre; and 1 of toxaphene, 2 lb. per acre. Because pretreatment sampling revealed no significant variation in infestation between plots, percentage control was based on differences in cutworm counts between treated and untreated plots 8 and 16 days after treatment. Density of cutworms was determined by examining five randomly selected 1-square-foot areas to a depth of approximately 4 inches in each plot.

Sprays were made from emulsifiable concentrates and applied with a power sprayer mounted on a small tractor. Fifteen gallons of spray under a pressure of 50 lb. p.s.i. were applied per acre. Fan-spray nozzles at boom height of 21 inches gave complete coverage of ground. Application of insecticides was made on May 4, 1955 when the wheat was 6 to 8 inches high and in the joint stage. Wind was estimated at 0 to 2 m.p.h. Soil was loose and dry down to at least 4 inches. Cutworms were mainly fifth and sixth instars based on measurements of head capsules (Parker *et al.* 1921). Weather data between treatment and initial sampling of control results 8 days afterwards were the following. Temperature: range maximum 60° to 83° F.; mean maximum 73° F.; range minimum 31° to 47° F.; mean minimum 37° F. Precipitation: 0.10 inch falling on the fifth day after treatment. Weather data between treatment and the final sampling at 16 days were the following. Temperature: range maximum 51° to 83° F.; mean maximum 72° F.; range minimum 31° to 49° F.; mean minimum 39° F. Precipitation: 1.83 inches.

Effectiveness of aircraft application of endrin for control of pale western cutworm was also investigated. For this test 3 blocks of 4 plots each were established. The individual plots measured 120×530 feet. Three treatments and one check were assigned at random to each block. Treatments consisted of endrin at 0.1, 0.2, and 0.3 lb. per acre. Because pretreatment sampling revealed no significant variation in infestation between plots, percentage control was based on differences in cutworm counts between treated and untreated plots 12 and 20 days after treatment. Density of cutworms was determined by examining 10 randomly selected 1-square-foot areas to a depth of approximately 4 inches in each plot.

Emulsion sprays were applied from a Stearman plane equipped with a boom which bore 21 nozzles arranged as in the diagram on page 21 of Farmers' Bulletin No. 2062 of the U. S. Department of Agriculture. The rate of application was 1 gallon per acre. Fan-spray nozzles No. 80.06 were turned 45° rearward. Pump pressure was maintained at 35 lb. p.s.i. and air speed ranged from 90 to 95 m.p.h. The plane was flown about 8 feet above ground. Treatments were made on May 12, 1955 when the wheat was in the joint stage. Wind measured 10 m.p.h. and blew down the length of the plots at an angle of approximately 25° causing the spray to drift somewhat on adjoining lee plots. Because of this drift, mortalities in two of the check plots were significant on the 20th day after treatment. The third check plot which was up wind and unaffected by drift was used for calculating percentage control obtained at the time of final sampling. Cutworms were mainly fifth and sixth instars based on measurements of head capsules.

Weather data between treatment and initial sampling of control results at 12 days afterwards were the following. Temperature: range maximum 51° to 83° F.; mean maximum 73° F.; minimum range 32° to 49° F.; mean minimum 41° F. Precipitation: 1.93 inches. Weather data between treatment and final sampling at 20 days afterwards were the following. Temperature: range maximum 51° to 83° F.; mean maximum 71° F.; range minimum 32° to 52° F.; mean minimum 41° F. Precipitation: 4.27 inches.

In addition to the above tests, results of applying en-

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Table 1.—Control of pale western cutworm in wheat with ground rig. Pine Bluffs, Wyoming. May, 1955.

Treatment	Pounds Actual Toxicant per Acre	Mean No. Cutworms per Sq. Foot		Per Cent Reduction	
		8 Days	16 Days	8 Days	16 Days
Endrin	0.4	0.0	—	100	—
Endrin	0.3	0.4	0.0	91	100
Endrin	0.2	0.7	0.0	85	100
TDE	2.0	0.4	0.1	91	98
Dieldrin	0.5	1.2	0.2	73	94
TDE	1.0	1.3	0.2	70	94
Endrin	0.1	2.0	0.3	54	92
Dieldrin	0.4	2.2	0.5	50	84
Heptachlor	0.5	2.7	1.5	38	55
Toxaphene	2.0	4.0	2.5	9	24
Untreated		4.4	3.3	0	0
L.S.D. at 5% level		1.8	1.2		
at 1% level		2.4	1.7		

drin commercially by airplane at 0.2 lb. per acre in 1 gallon of spray were followed in six different fields. Two fields were treated on May 10, 1955 when the wheat was in the joint stage and cutworms mainly in the fifth and sixth stadia. Four fields were treated on May 29 and May 30 when the wheat was in the boot stage and cutworms mainly in the seventh and eighth stadia. One check field was employed for comparison with the two fields treated on May 10 and another check field for comparison with the four fields treated on May 29 and 30. The check fields were located in the same areas as the treated fields. Endrin was applied in diesel fuel on two fields treated May 29; on all the other fields endrin was applied in emulsions.

RESULTS.—Table 1 summarizes the ground rig test. Endrin treatments of 0.2, 0.3, and 0.4 lb. per acre provided complete control as they reduced cutworm densities to zero. Not greatly inferior nor significantly different were the reductions of cutworms by endrin at 0.1 lb., dieldrin at 6 oz. and 0.5 lb., and TDE at 1 and 2 lb. per acre. Control of cutworms was significantly inferior with heptachlor at 0.5 lb. and toxaphene at 2 lb. per acre.

Table 2 summarizes the results obtained by airplane applications of endrin. No significant differences in effectiveness of control followed the application of endrin at the three rates of 0.1, 0.2, and 0.3 lb. per acre, but like the ground rig tests 0.2 and 0.3 lb. of endrin per acre reduced cutworm densities to zero, whereas 0.1 lb. per acre failed to give complete kills.

Table 2.—Effectiveness of endrin[a] against pale western cutworm. Pine Bluffs, Wyoming. May, 1955.

Treatment	Pounds Actual Toxicant per Acre	Mean No. Cutworms per Sq. Foot		Per Cent Reduction	
		12 Days	20 Days	12 Days	20 Days
Endrin	0.3	0	—	100	—
Endrin	0.2	0.1	0	96	100
Endrin	0.1	0.2	0.06	91	97
Untreated		2.5	2.0	0	0
L.S.D. at 5% level		0.7			
at 1% level		1.1			

[a] Applied by airplane delivering 1 gallon of emulsion per acre.

Table 3.—Commercial airplane applications of endrin[a] against pale western cutworm. Laramie County, Wyoming. 1955.

Date of Application	Instar of Cutworms	Stage of Wheat	Number Cutworms per Square Foot				
			Pretreatment	9 Days	13 Days	16 Days	22 Days
May 10	5 & 6	Joint	2.9		0.0		0.0
May 10	5 & 6	Joint	7.5		0.1		0.0
Check	5 & 6	Joint	4.0		3.1		2.0
May 29	7 & 8	Boot	3.5	0.7		0.3	
May 29	7 & 8	Boot	2.9	1.0		0.3	
May 30	7 & 8	Boot	3.4	0.8		0.3	
May 30	7 & 8	Boot	3.3	1.5		0.9	
Check	7 & 8	Boot	3.5	2.9		2.1	

[a] Applied at 0.2 pound per acre in 1 gallon of spray.

Table 3 shows the results obtained by commercial airplane applicators in using endrin at 0.2 lb. per acre. When treatments were applied early in May at approximately the same time as treatments in the above tests, cutworm densities were reduced to zero; but when treatments were made in late May against older instars, cutworms were not completely eradicated.

DISCUSSION.—The first indication that the pale western cutworm could be controlled with insecticidal sprays was revealed in the tests of Jacobson *et al.* (1952). They applied six different chlorinated hydrocarbons with a ground sprayer delivering 4 gallons of spray per acre and obtained, 14 days after treatment, reductions of 81% with 0.5 lb. of dieldrin per acre and 82% with 2 lb. of chlordane per acre. Poor results were obtained with aldrin, BHC, toxaphene, and heptachlor. The present tests corroborate their data on dieldrin, toxaphene, and heptachlor. Hoerner (1953) tested eight different chlorinated hydrocarbons and showed that in addition to dieldrin, TDE at 1.5 lb. per acre was effective in controlling pale western cutworms. He further showed that when aldrin or heptachlor at 0.5 lb. per acre was worked into the soil each of these was also effective in controlling this species.

An indication that volume of spray per acre influences effectiveness of treatment is found in the results from Hoerner's plots in which soil was unworked after treatment. Thirteen gallon volumes of dieldrin, aldrin, or chlordane sprays provided better kills than 7 gallon volumes, in spite of the fact that the amount of toxicant per acre remained the same. Such was not the experience in the present tests with endrin. Endrin at 0.1, 0.2, or 0.3 lb. per acre was as effective when applied in 1 gallon of spray by airplane as in 15 gallons of spray by ground rig. Having an effective insecticide which aircraft can apply at low gallonages is advantageous since large acreages can be treated in a short period of time. According to Jacobson *et al.* (1952), it is not generally feasible to determine before damage is evident whether or not pale western cutworms are present in damaging numbers. They suggest instead that wheat growers take prompt action at the first evidence of crop thinning by the cutworms.

Hoerner's study also indicated that working of the soil with a spike toothed harrow or a tine weeder after spray-

ing increased the effectiveness not only of aldrin and heptachlor but also of dieldrin and TDE. This operation has the disadvantage of increasing cost of control. Since endrin reduced the cutworm densities to zero without working of the soil, the additional operation seems unnecessary.

The reason for the failure of endrin at 0.2 lb. per acre to reduce cutworm populations to zero when treatments were made in late May is unknown, but three possible reasons may be suggested. First, cutworms may not have been as susceptible to the insecticide. Second, the older larvae may have ceased feeding and may have been entering the period of summer dormancy. And third, the wheat was taller and denser, perhaps allowing less of the insecticide to cover young tillers being fed upon. Whatever the reason, control measures applied in late May did not prevent much damage and were probably unprofitable.

SUMMARY.—Endrin, dieldrin, TDE, heptachlor, and toxaphene were tested for the control of the pale western cutworm, *Agrotis orthogonia*, infesting winter wheat. Endrin at 0.2, 0.3, or 0.4 lb. per acre applied by a ground rig delivering 15 gallons of spray per acre gave complete control, as densities of cutworms were reduced to zero. Endrin at 0.1 lb., TDE at 1 and 2 lbs., and dieldrin at 6 oz. and 0.5 lb. per acre provided cutworm reductions not greatly inferior nor significantly different, but control with heptachlor at 0.5 lb. and toxaphene at 2 lb. was significantly inferior. Endrin at 0.2 and 0.3 lb. per acre applied by aircraft as 1 gallon emulsions also reduced cutworm densities to zero.

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Identification of the Sulfoxide and Sulfone Plant Metabolites of the Thiol Isomer of Systox[1,2]

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Previous studies (Fukuto *et al.* 1955, March *et al.* 1955) describing the chemical behavior of the Systox[3] isomers, *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thiono isomer) and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer) in biological systems, indicated that these isomers were converted to certain oxidation products that were even more potent inhibitors of cholinesterase than the parent isomers. Comparison of cholinesterase inhibitory activity, systemic activity, mammalian and insect toxicities, and relative behavior on paper chromatograms of the metabolic and synthetic oxidation products showed that each isomer was first converted to the corresponding sulfoxide by oxidation of the ethylmercaptoethyl moiety, and suggested that these sulfoxides were subsequently converted to the sulfones. As final proof in establishing the absolute identity of these metabolic products, it was decided to inspect their infrared spectra and compare them with the synthetic oxidation products.

Previous studies by Metcalf *et al.* (1954) have shown that the thiol-isomer metabolites accumulate in plants from 5 to 10 times as rapidly as the thiono-isomer metabolites and are considerably more persistent. For this reason, and because of the much greater importance of the thiol isomer, it was decided to investigate the thiol isomer first.

Metabolites of both thiono and thiol isomers have been isolated on sheets of paper impregnated with silicone or propylene glycol, by using the appropriate mobile solvent (Fukuto *et al.* 1955), but it has not been possible to isolate enough of either metabolite by this method for infrared analysis. Isolation by the use of the charcoal-celite column in column chromatography was found to give the best separation of the thiol-isomer metabolite with the least amount of plant contaminant. By this method the major metabolic product from the thiol isomer, after 4 to 6 days in the cotton plant, was identified as *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (sulfoxide of thiol isomer), which was further metabolized to *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (sulfone of the thiol isomer).

MATERIALS AND METHODS.—P^{32} radiotracer of *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate was used in this investigation. Red phosphorus (4.0 gm. Baker and Adamson amorphous red powder) sealed in a quartz ampule was service-irradiated in the neutron pile at Oak Ridge, Tennessee, for 4 weeks. The calculated activity of the radioactive phosphorus was approximately 200–300 mc.

P^{32}-Labeled Diethoxythiophosphoryl Chloride.—In a 50-ml. 3-necked flask equipped with stirrer, dropping funnel, and condenser was placed 3.0 gm. P^{32}-red phosphorus, 7.7 gm. sulfur, and 1.3 gm. phosphorus pentasulfide. Dry

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[2] Part V of a series of papers on the mode of action of Systox. This work was supported in part by grants from the U. S. Atomic Energy Commission, Contract AT(11-1)34, Project 6, and from the Chemagro Corporation, New York, N. Y.
[3] Trade-mark; Chemagro Corporation, New York, N. Y.

nitrogen was passed through the system, and the flask was heated in a silicone bath until the mixture melted. The mixture was then stirred until homogenous. The temperature of the bath was raised slowly to 280° C., maintained at that temperature for 5 hours, and then lowered to 80° C. After the addition of 15 ml. benzene, 12.7 ml. absolute ethyl alcohol was added dropwise. The mixture was heated at reflux until there was no further reaction (6 to 8 hours). The resulting dark solution was cooled in an ice bath, and 6 ml. of liquid chlorine was allowed to vaporize beneath the surface of the reaction mixture. The mixture was poured into cold water, and the product was extracted into benzene. The benzene solution was dried over anhydrous sodium sulfate, and the P^{32}-labeled diethoxythiophosphoryl chloride was distilled; b. p. 70° C. (5.0 mm.); yield, 8.2 gm.

P^{32}-labeled *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate was prepared as described by Fukuto & Metcalf (1954), from 2.2 gm. diethoxythiophosphoryl chloride and 1.25 gm. 2-hydroxydiethyl sulfide, and was converted to *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer) by heating at 120–130° C. for 4 hours.

The synthesis of *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (thiol-isomer sulfoxide) and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (thiol-isomer sulfone) has been described by Fukuto *et al.* (1955).

Isolation and Identification of Thiol-Isomer Metabolites from the Cotton Plant.—The bases of approximately 50 young cotton plants were treated with 5 to 15λ (total of 700 mg.) of P^{32}-labeled thiol isomer. After 4 to 6 days the leaves (200 gm. total) were divided into 4 equal aliquots and each was homogenized with 250 ml. water in a Waring Blendor for 5 minutes. The aqueous part of the homogenate was separated by centrifugation, and the clear supernate was extracted with three 115-ml. portions of chloroform. The leaf residue was rehomogenized with 150 ml. chloroform, filtered, and the filtrate was combined with the other chloroform fractions. After this treatment the amount of radioactivity remaining in the leaf material was negligible. The chloroform solution containing the thiol-isomer metabolite was concentrated to about 25 ml. and was added to the charcoal column without further treatment.

The charcoal chromatographic column was prepared as follows. A mixture of charcoal and concentrated hydrochloric acid was boiled for 10 minutes and then washed repeatedly with water until the wash was neutral to litmus. It was diluted with one-half its volume of celite, slurried in hexane, and poured into a column (35×600 mm.). The packed column was washed with 200 ml. *n*-hexane, 200 ml. absolute ethyl alcohol, and 100 ml. *n*-hexane in the order named. The plant metabolite sample was added to the column and eluted, first, with 2 l. of a solvent consisting of (by volume) 70 parts *n*-hexane, 23 parts chloroform, and 7 parts absolute ethyl alcohol, and then with 1.5 l. of a solvent consisting of 60 parts *n*-hexane, 30 parts chloroform, and 10 parts absolute ethyl alcohol. The eluate fractions were collected in 50-ml. portions.

It was found that this charcoal column (and several other types tried) could not completely separate the thiol-isomer metabolite from plant material. In particular, a compound which had a strong carbonyl absorption band (1730 $cm.^{-1}$), and which interfered by absorbing in the P=O (1265 $cm.^{-1}$), P—O—C (1020 $cm.^{-1}$), and sulfoxide (1050 $cm.^{-1}$) regions, was found in the fractions containing radioactivity. This substance was separated from the radioactive metabolite in the following manner. The eluate fractions that contained radioactivity and which came from a solid band on the column were combined and concentrated to an oily residue. This residue was taken up in 100 ml. water and washed with one 50-ml. and two 25-ml. portions of benzene, the radioactivity remaining almost entirely in the aqueous phase. The metabolite was then extracted from the aqueous phase with two 50-ml. portions of chloroform and was not contaminated with plant material. The chloroform solution was dried over sodium sulfate, concentrated to an oil *in vacuo*, and the infrared spectrum of the oil was determined in a Perkin-Elmer model 21 self-recording infrared spectrophotometer, using sodium chloride optics and carbon tetrachloride as a solvent.

Essentially the same experimental procedure was used in the isolation of the thiol-isomer sulfone. The bases of young cotton plants were treated with 5 to 15λ of P^{32}-labled thiol-isomer sulfoxide (total of 500 mg.), and the leaves were harvested 2 weeks after application. The metabolite was isolated by the chromatographic procedure described above. In the solvent partition purification procedure, diethyl ether was used instead of benzene to remove the plant material from the aqueous phase previous to extraction into chloroform.

DISCUSSION OF RESULTS.—The major thiol-isomer metabolite from the cotton plant 4 to 6 days after application, which constituted 98.5 per cent of the total radioactivity recovered, was separated on the column in a sharp band. This is shown by the large peak in figure 1, in which radioactivity is plotted against eluate fractions. A small peak found at eluate fraction 30 amounted to 1.5 per cent of the total radioactivity. By integrating the area of the two peaks, the total weight of the metabolic products, calculated as the thiol isomer, was found to be 200 mg. This is 29 per cent of the amount applied and is in the proper order of magnitude, since losses could not be avoided in the extraction process and only leaves were harvested, leaving a considerable quantity of radioactivity in the stem and at the point of application.

The infrared spectra of the major metabolite and the synthetic thiol-isomer sulfoxide (Fig. 2) are essentially identical and prove, beyond doubt, that the two compounds are the same. The pertinent absorptions for the P=O (1265 $cm.^{-1}$), P—O—CH_2CH_3 (1160 $cm.^{-1}$), S—O (1050 $cm.^{-1}$), and P—O—C (1020 $cm.^{-1}$) moieties are present in both spectra (Bellamy 1954).

Determination of the infrared spectrum of the second small peak was attempted, but there was not enough of the metabolite for analysis, and the spectrum was further complicated by the contamination of plant material.

In earlier work Fukuto *et al.* (1955) found that a few of the chromatograms (propylene glycol paper) of the thiol-isomer metabolites from the cotton plant gave a second spot with an R_f value slightly greater than that of the major metabolite (thiol-isomer sulfoxide), and which comprised only a small percentage of the total metabolic products. The thiol-isomer sulfone was suggested as the

PER CENT TRANSMISSION

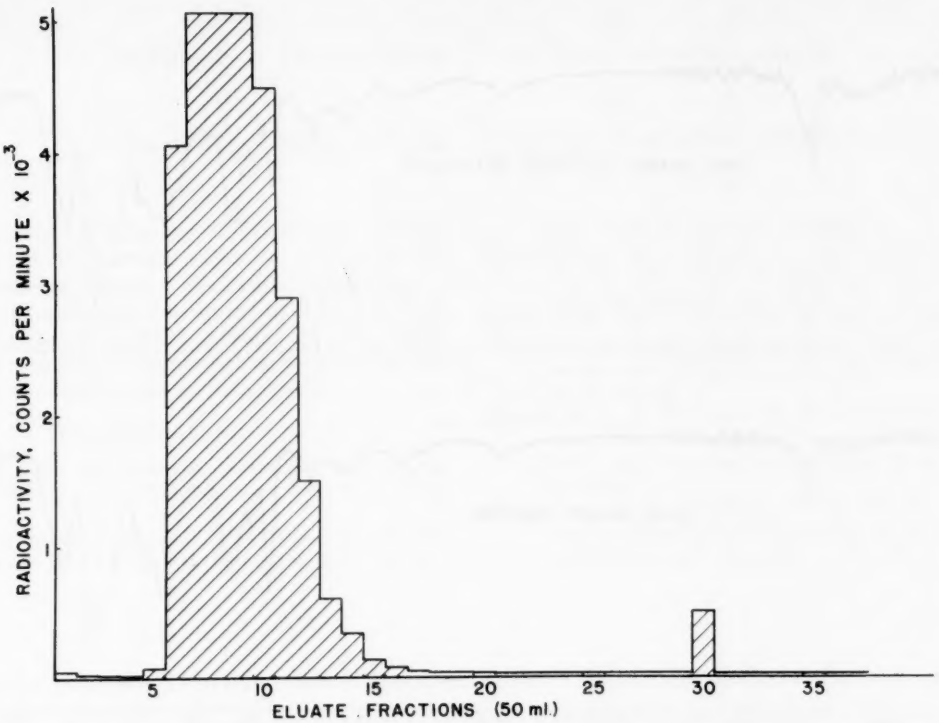


FIG. 1.—Profile of chromatogram of metabolites from the cotton plant 4 to 6 days after application, showing plot of radioactivity vs. eluate fractions.

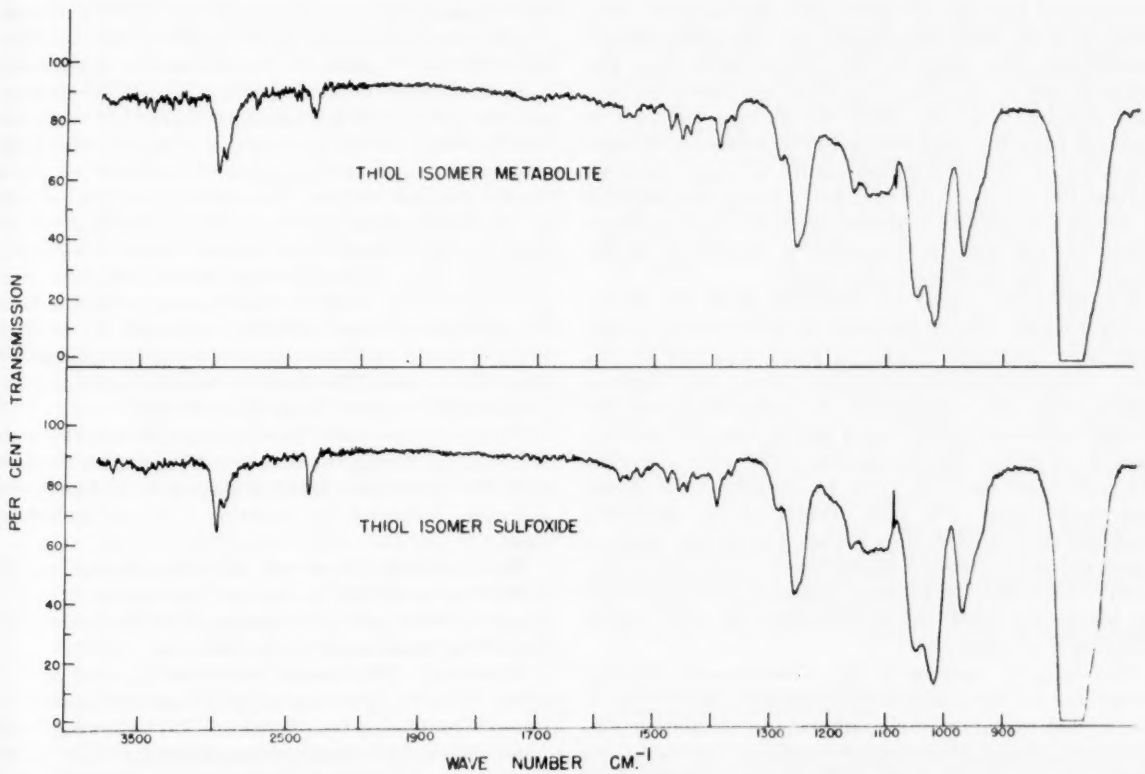


FIG. 2.—Infrared spectra of major thiol-isomer metabolite and thiol-isomer sulfoxide.

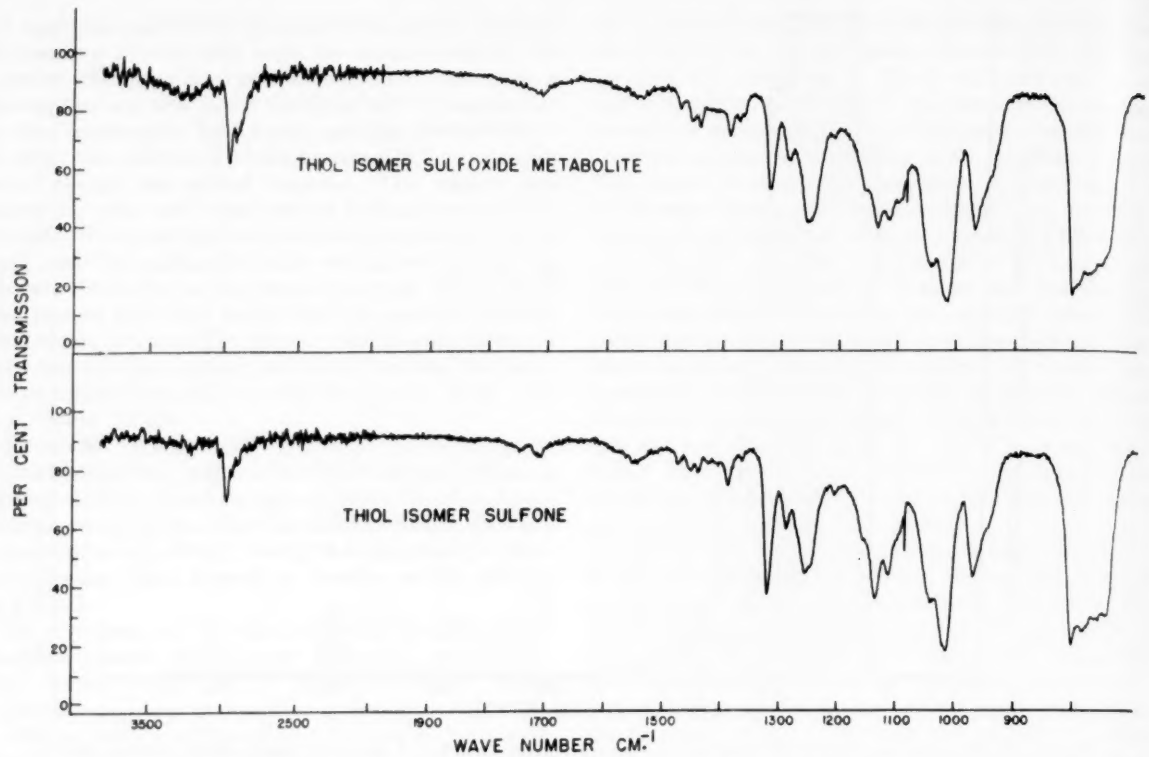


FIG. 3.—Infrared spectra of thiol-isomer sulfoxide metabolite and thiol-isomer sulfone.

structure of this second metabolite. Subsequent work (Metcalf *et al.* 1956) has shown that this metabolite is considerably less stable in the cotton plant than the sulfoxide and is therefore somewhat less persistent and more difficult to isolate. Since the natural sequence of reactions from the thiol isomer to the sulfone is through the sulfoxide, P^{32}-labeled thiol-isomer sulfoxide was used to treat the cotton plants in order to insure the isolation of enough metabolite for infrared analysis in the minimum length of time and to eliminate the possibility of the presence of any unchanged thiol isomer.

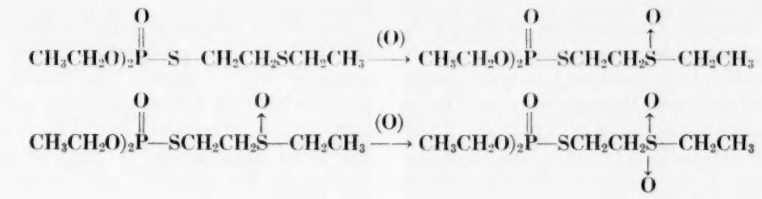
The radioactive products extracted from the cotton leaves 2 weeks after application of the thiol-isomer sulfoxide were separated from the plant material by the same chromatographic procedure, using the charcoal column. The total radioactivity was recovered from the column in a sharp band, and a plot of the radioactivity against the eluate fraction gave a profile similar to that of the sulfoxide shown in figure 1, except that the second peak was absent. The total weight of the metabolic products, calculated as thiol isomer, was 25 mg. which is 5 per cent of the amount applied and is a reasonable recovery, since the thiol-isomer sulfoxide is absorbed by the plant at a much slower rate than the thiol isomer (Metcalf *et al.* 1956).

The infrared spectra of the thiol-isomer sulfoxide metabolite and the synthetic thiol-isomer sulfone (Fig. 3) proves that oxidation of the sulfoxide to the sulfone also occurs in plants. Pertinent absorptions for the P=O (1265 cm.$^{-1}$), P—O—C_2H_5 (shoulder at 1160 cm.$^{-1}$) P—O—C (1020 cm.$^{-1}$) and —SO_2— (1320 and 1140 cm.$^{-1}$) moieties are present in both spectra. The relatively smaller value of the ratio of the heights of the 1320 cm.$^{-1}$ and 1265 cm.$^{-1}$ peaks in the metabolite spectrum, in comparison with the similar ratio in the sulfone spectrum, and the presence of a peak at 1050 cm.$^{-1}$ (S→O), show that the eluate from the column contains a considerable amount of unchanged thiol-isomer sulfoxide as well as the thiol-isomer sulfone. The oxidation of the sulfoxide to the sulfone apparently proceeds at a much slower rate than the transformation of the thiol isomer to the sulfoxide in the plant. The slight shoulder at 1050 cm.$^{-1}$ in the spectrum of the synthetic thiol-isomer sulfone indicates the presence of some sulfoxide, although the elemental analysis for the substance was in good agreement with the theoretical values. This shoulder was also found in sulfone preparations received from other sources.

These spectra prove that the principle reaction in the cotton plant during the first 4 to 6 days after treatment with the thiol isomer is the oxidation to the thiol-isomer sulfoxide illustrated by equation 1, given on the next page.

The sulfoxide, however, is subsequently oxidized further to the sulfone, as depicted by equation 2, and this process is relatively slow, compared to the initial oxidation of the thiol isomer to the sulfoxide.

SUMMARY.—The major metabolic product of *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer) from the cotton plant 4 to 6 days after application was isolated by column chromatography. The infrared spectrum of this metabolite proved conclusively that it was identical with *O,O*-diethyl *S*-ethyl-2-sulfinylethyl



phosphorothiolate (thiol-isomer sulfoxide). The infrared spectrum of the thiol-isomer sulfoxide metabolite isolated from the cotton plant 2 weeks after application of the sulfoxide proved that subsequent oxidation to *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (thiol-isomer sulfone) occurs at a somewhat slower rate than the conversion of the thiol isomer to the sulfoxide.

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Azobenzene-Aramite and Azobenzene-Chlorobenzilate Sprays for Resistant Spider Mites on Greenhouse Roses[1]

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The most serious problem of greenhouse roses is the control of resistant strains of the two-spotted spider mite, *Tetranychus telarius* L. Azobenzene was used extensively following the work of Blauvelt (1945), the usual method of application being vaporization of the material from steam pipes. Pressure fumigators and candles were also used (Blauvelt 1947b). While azobenzene fumigation occasionally caused some foliage damage, its main disadvantage was a bleaching of flowers of pink and red varieties (Blauvelt 1947a), and for this reason it was seldom used after TEPP and parathion became available.

In southern California resistance to parathion and other organic phosphorus compounds developed about 1949, and to Aramite in 1952. When field observations in the spring of 1955 indicated that the mites had become resistant to Chlorobenzilate, experiments with azobenzene sprays were begun. While Huckett (1948) obtained excellent control of the two-spotted spider mite with azobenzene sprays, as far as the writers have been able to ascertain, they have not previously been used on greenhouse roses.

MATERIALS AND METHODS.—The materials used were azobenzene[2], Chlorobenzilate[3] and two formulations of Aramite.[4] These were applied at the following rates per 100 gallons of water: 3 pounds of 70% azobenzene wettable powder; 1½ pounds of 70% azobenzene wettable powder plus ¾ pound of 25% Chlorobenzilate wettable powder; 1½ pounds of 70% azobenzene wettable powder plus ¾ pound of 15% Aramite wettable powder; and ¾ pound of 15% Aramite wettable powder plus ¾ pound of 25% Chlorobenzilate wettable powder.

The experiments were conducted in three commercial rose ranges. In the preliminary tests, some of the sprays were applied by the growers and some by the writers. All sprays were applied with power equipment and directed at the undersides of the leaves. Range 1 and range 2 were equipped with stationary spray systems with pumps having a capacity of 10 gallons per minute and 1000 pounds pressure.[5] In these ranges a pressure of approximately 450 pounds was maintained at the pump and the sprays were applied with spray rods having a single nozzle with a TeeJet tip.[6] Range 1 used a No. 8006 tip in all sprays; in Range 2 a No. 8004 tip was used in the July sprays and a No. 8002 tip in the September and November sprays. Range 3 had a 50-gallon Bean Estate Sprayer with a capacity of 4 gallons per minute and 400 pounds pressure. In the first tests at this range two sprays rods with Bean hollow cone nozzles were used from the rig. In one test the spray was applied by the writers with another sprayer using a spray rod with a No. 8006 tip and a pressure of 450 pounds.[5] All subsequent sprays were applied with the Bean rig at a pressure of 350 to 400 pounds and using a single spray rod with a No. 8006 tip.

In all experiments, both preliminary and large-scale, the effectiveness of the sprays was checked by the writers 7 to 14 days after application, and a continual check of the results was made by the growers.

PRELIMINARY TESTS.—Excellent control was obtained

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[2] Azofume-70, Plant Products Corp.
[3] Chlorobenzilate 25W, Geigy Chemical Co.
[4] Ortho-Mite 15W (Florida formulation), California Spray Chemical Corp.; and Niagaramite 15 Spray, Niagara Chemical Division, Food Machinery Corp.
[5] Gage Pump Co., Bell, Calif.
[6] Spraying Systems Co.

with the azobenzene-Aramite combination in Range 1, and with the azobenezene-Chlorobenzilate combination in Range 2. One week after treatment no live mites could be found in the sprayed plots and the eggs appeared to be drying up. In both ranges azobenzene at 3 pounds of the 70 per cent powder per 100 gallons failed to kill all of the active stages. In Range 1, the Aramite-Chlorobenzilate combination did not give satisfactory control.

In Range 3, poor control was obtained by the grower with both the azobenzene-Aramite and azobenzene-Chlorobenzilate combinations. That this was due to poor coverage was demonstrated when the writers sprayed one house with azobenzene-Aramite combination on September 30 and the following day the grower sprayed another house. Examination on October 7 showed that excellent control had been obtained in the house sprayed by the writers but not in the one sprayed by the grower.

RESULTS.—In *Range No. 1.*—In this range, containing 100,000 plants, control with Chlorobenzilate sprays during the spring was unsatisfactory. Following the successful preliminary tests with the azobenzene-Aramite spray, the entire range was sprayed with this combination July 12 to 15. This was a very thorough application, and was followed by a second spray July 21 to 22. It had been planned to apply two more sprays in August in an attempt to eradicate the mites, but because of a shortage of azobenzene this was not possible, and individual houses were sprayed as infestations appeared. During the period August 19 to September 30 the range received the equivalent of one complete spray. There were 22 houses and, of these, 4 were not sprayed at all, 13 received one spray and 5 received two sprays. Of the seven houses in which the most frequent and heaviest infestations usually occurred, only one received two sprays. A third complete spray was applied October 28–November 5 after the pinch for the Christmas crop.

Outstanding control was obtained with the azobenzene-Aramite sprays. About a month after the second spray a few mites began to appear. These were scattered and not necessarily in the areas in which the worst mite infestations usually occurred. Since this grower has a field of roses adjacent to the range, some of these infestations may have resulted from mites brought in on the hands or clothes of workers. Infestations at the time of the last spray were light and scattered. In an extensive examination on December 7, only two infested leaflets were found in the range, and no further spraying was required for the Christmas crop.

Range No. 2.—Range 2 contained 29,000 plants. Control in the spring with Chlorobenzilate sprays was very poor, indicating that the mites had become quite resistant.

The first azobenzene-Chlorobenzilate spray was applied in two parts. A house of heavily infested Snow White variety was sprayed on July 19. Examination a week later showed that an excellent kill had been obtained. The remainder of the range was then sprayed with the exception of part of one house, which was left due to a shortage of azobenzene. The second, third and fourth sprays were applied September 12 to 14, September 21 to 23 and November 7 to 11, respectively. In the last application, the amount of Chlorobenzilate wettable powder was reduced from 12 to 10 ounces per 100 gallons.

Control of the mites in this range was excellent. Following the first spray no live mites were found in the sprayed areas for over a month. The fourth spray, applied after the pinch for the Christmas crop, was mainly a preventive measure, since no mites had been found in the range since the third application. A final check was made by the writers on December 7, and except for spot spraying of one area which apparently had been missed in the November application, no more sprays were applied until after the Christmas crop.

Range No. 3.—Range 3 contained 110,000 plants. While poor control was obtained during the summer with either Aramite or Chlorobenzilate sprays, this may have been due to some extent to poor coverage. Following the excellent control obtained by the writers with the azobenzene-Aramite spray applied on September 30, the grower tested this combination more extensively during October in the worst areas in the range. Control in these tests ranged from fair to good. During this period a new spray crew was being trained, and as the men became more experienced, coverage was greatly improved with a consequent increase in control.

Between November 2 and November 25 the range received two complete sprays with the most heavily infested areas receiving a third. Examination of the range on December 5 showed that very good control had been obtained as only a few mites in several isolated spots were found. By December 13, however, enough mites were found in two houses to require some spot-spraying before Christmas.

Other Ranges.—Excellent control of the two-spotted spider mite has been obtained with commercial applications of azobenzene combination sprays in two other rose ranges. In one southern California range, the azobenzene-Chlorobenzilate combination was used with less foliage damage than with Chlorobenzilate alone. The change was made principally to reduce injury as the mites had shown no indication of resistance to Chlorobenzilate.

The azobenzene-Aramite combination was used with excellent results in one large range in the San Francisco Bay Area in which the mites were reported to be quite resistant to Aramite. In this case the amount of 15% Aramite was increased to 1 pound of the wettable powder per 100 gallons and 2 ounces of Triton B-1956 was added. Small-scale tests were run in two other ranges in the San Francisco Bay Area with the azobenzene-Aramite combination and excellent control was obtained.

Spray Damage.—Azobenzene combination sprays have been extensively used in five ranges with only very minor damage, most of which is attributed to either the Aramite or the Chlorobenzilate.

Foliage damage has been a slight crinkling or curling of new growth on sensitive varieties such as Rosen Elfe, Carol Amling and Pink Bountiful. When the "Florida formulation" of Aramite was used, this crinkling was more pronounced. However, serious damage has been reported from the use of the "Florida formulation" at dosages of 1½ pounds of the 15 per cent powder per 100 gallons. In the case of the azobenzene-Chlorobenzilate combination, both ranges using it have had less foliage damage than that normally resulting from the use of the Chlorobenzilate alone at 1 pound of the 25 per cent pow-

der per 100 gallons. In Range 2, no foliage damage at all resulted from the November spray in which the amount of Chlorobenzilate was reduced from 12 to 10 ounces.

In Range 1, a slight bleaching of the upper surface of mature leaves occurred during the fall. Since none had been observed following sprays applied during the warm summer months, the grower attributed it to cooler weather and lower humidity. Whether this bleaching was caused by the azobenzene remains to be determined, since similar symptoms have been observed on roses in the Entomology greenhouse at U.C.L.A. which had never been sprayed. The same symptoms were also found at Range 3 and the grower stated that they had been observed before the azobenzene sprays were used. A possible cause of this bleaching is smog (air pollution).

Bleaching of the flowers does not appear to be the serious problem with azobenzene combination sprays that it was with azobenzene fumigation. In the five ranges which have extensively used the sprays, bleaching has been reported from two. In Range 3, slight bleaching of Better Times occurred but Red Delight and other varieties were not affected. Bleaching of Red Delight and one or two other varieties was reported from the range in the San Francisco Bay Area. No bleaching at all was observed in the other three ranges.

In only one range has more than minor damage occurred from the use of azobenzene combination sprays. This was in one of the ranges which tested the azobenzene-Aramite spray on a small scale. Foliage damage was principally a bleaching of mature leaves and a marginal scorch of tender young growth. Severe bleaching of the flowers also occurred on some varieties. This range is located in a peculiar climatic area and its roses apparently have been more sensitive to spray injury than those of other ranges. In this case, smog may also have been a factor, as some damage occurred to flowers of plants that were not sprayed.

Varieties of roses in Ranges 1, 2, and 3 included Better Times, Carol Amling, Fashion, Garnette, Golden Rapture, Happiness, Peter's Briarcliffe, Pink Bountiful, Pink Delight, Pink Fill, Pink Garnette, Pinnochio, Red Delight, Rosalind Russell, Rosen Elfe, San Fernando, Snow White, Yellow Gloria, and Yellow Polly.

DISCUSSION.—When these experiments were begun, the use of azobenzene as a spray seemed to offer several advantages over fumigation or dust applications. The work of Blauvelt & Hoffman (1947), Hamilton (1947) and English (1950) indicated that azobenzene was most effective as an ovicide, and as a spray it could be more readily combined with other materials for a better kill of the active stages. It was also felt that there was a possibility of less bleaching of the flowers than with fumigation, and that better coverage could be obtained with sprays than with dusts.

Blauvelt & Hoffman (1947) found that fumigation with a combination of azobenzene and HETP was more effective against all stages of the mites than either material used alone. English (1950) obtained the same results against parathion-susceptible mites but not against parathion-resistant mites when azobenzene was used as a supplement to parathion in aerosols. In these experiments Aramite and Chlorobenzilate were used as supplements to the azobenzene and both increased the effectiveness of the azobenzene against the active stages of Aramite- and Chlorobenzilate-resistant mites.

The azobenzene-Aramite and azobenzene-Chlorobenzilate sprays killed all stages of the mites but were slow acting. There was almost no mortality of the active stages during the first 2 days and a complete kill was not obtained until the fourth or fifth day. During this period the females apparently laid no eggs, or else the eggs failed to hatch. Examinations 7 to 10 days after application showed the mites dead with the eggs discolored and drying up. Almost invariably when live mites were found the eggs also were unaffected, indicating that coverage was poor or that the leaf had been missed. From this it would also appear that the azobenzene acted as a contact insecticide and not as a fumigant.

The importance of adequate spray equipment and thorough coverage was shown in Range 3. During the fall as the crews concentrated on better coverage, control greatly improved but never quite reached that obtained in Ranges 1 and 2. All of the spot spraying required in December was in varieties with very dense foliage while heavily infested areas in other varieties apparently had been completely cleaned up. This probably was due to the spray equipment, the pump lacking sufficient pressure to drive the spray through, and turn the leaves, in the denser foliage.

Percentage mortality counts were not made in determining the effectiveness of the sprays since, with good coverage, any spray that fails to give a complete kill of the mites is not satisfactory. While complete eradication is difficult in a large range, from the results obtained with the azobenzene combination sprays, the growers in Ranges 1 and 2 feel that it can be accomplished. In this respect, unless eradication is obtained, it can be assumed that resistance to the azobenzene combination sprays will soon develop.

SUMMARY.—Excellent control of resistant strains of the two-spotted spider mite on greenhouse roses was obtained with combination sprays of azobenzene-Aramite and azobenzene-Chlorobenzilate. Bleaching of the flowers did not appear to be the serious problem it had been with azobenzene fumigation; of five ranges which extensively used the sprays, three reported that no bleaching occurred.

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A Winter Survey of Coleoptera in Feed and Flour Mills[1]

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Cotton & Wagner (1935) reported that in general the insect population of mills is small in winter. They found that the number of insects increased rapidly in spring and then decreased to a low ebb in July because of fumigations carried out in early summer. They found a rapid rise in numbers after fumigation until fall, when a decline in numbers set in with the advent of cold weather. They also noted that mills under continuous operation 24 hours per day could have heavy insect infestation during the winter.

Good (1937) studied for an entire year insect populations in the mill streams of 19 mills within 200 miles of Independence, Kansas. He made no collections in February and the November and December collections were considered as one, since some of the mills were sampled in November and the rest in December. He did not identify *Tribolium*, *Laemophloeus*, *Palorus*, or *Alphitobius* to species.

Apparently no previous work dealing with the winter incidence of insects in the Buffalo area had been done, and since this region is a leading milling area and since it is still the custom in many mills to relax insect control programs in winter, work was undertaken to determine the relative prevalence of species of Coleoptera occurring in mills during winter. Good (1937) had shown that Coleoptera made up 99.85% of all insects present in the flour mill streams that he studied.

Materials and Methods.—Collections were made at 11 feed and flour mills between October 12, 1954 and March 28, 1955. In each mill samples were taken from places that might be harboring insects. These samples consisted of whole grain, processed foods, floor sweepings, debris that had accumulated between floor boards and in the corners of rooms, grain dust on and within mill machinery, grain, flour, and dust at the base of chutes and other places peculiar to the mills investigated.

Results.—A total of 2632 insects representing 23 species were recovered from approximately 27 pounds of sample material. The species of insects found in each mill are recorded in table 1. None of the 23 species collected was found in each mill, but *Attagenus piceus* (Oliv.), the black carpet beetle, and *Oryzaephilus surinamensis*, (L), the saw-toothed grain beetle, were found in 8 of the 11 mills visited. These two species plus *Tenebroides mauritanicus* (L.), the cadelle, and *Tribolium confusum* Duv., the confused flour beetle, were the most prevalent beetles occurring in Buffalo mills during the colder months of the year. The temperatures of samples collected ranged from 3° C. to 26° C. *Tribolium confusum*, *Attagenus piceus*, *Gnathocerus cornutus* (F.), the broad-horned flour beetle, *Sitophilus oryza* (L.), the rice weevil, and *Tribolium castaneum* (Hbst.), the red flour beetle, in the above order of abundance, constituted the bulk of the insects collected.

Discussion.—Personnel of the mills visted were almost unanimous in the opinion that insects were scarce and reduced to an insignificant exiguity in mills during the winter. They felt that this was true for both heated and unheated mills. Our study showed that this opinion is not founded upon fact. The large number of beetles recovered shows that the insect population of mills may be quite high in winter when it is remembered that the amount of sample material examined was only a minute fraction of similar material present in the mills.

In regard to winter temperatures it was interesting to find specimens of *Oryzaephilus surinamensis* in a box car, the inside air temperature of which was only 4° C. The foreman stated that the fumigation of box cars had been discontinued the previous week as no grain insects were thought to be alive at this late date of the season.

That temperature has a marked affect upon the seasonal abundance of insects was noted by Wagner & Cotton (1935), and the effects of low temperatures have been investigated by several authors. Howe (1951) observed that the temperature in outbuildings at Buckinghamshire, England, seldom became cold enough to stop oviposition of the spider beetle, *Ptinus tectus*, and Howe & Burges (1951) found that *P. sexpunctatus* and *P. fur* survived the winter in unheated warehouses in Southern England. One of the two species of *Ptinus* collected in the Buffalo area was found in an unheated mill at 11° C., which agrees with the observations of Howe & Burges regarding this genus.

Several mill chemists interviewed said that spider beetles (Ptinidae) were quite numerous in the warmer months and were often found crawling on the outside of bags of feed and grain. This activity, however, is not limited to warm months only. Shepard (1939) stated that they have been seen crawling actively at 38° F. where other insects could not live successfully at such low temperatures.

Robinson (1926) found that *Sitophilus oryza* and *S. granarious* (L.), the granary weevil, became dormant at 7.2° C. and 1.6° C. respectively, and at those temperatures they could live for only about 18 days. Richards (1947) observed that hatching and larval development was extremely slow for these two species between 55° F. and 60° F. The above information was borne out in the present study by the fact that no specimens of *Sitophilus granarius* or *S. oryza* were collected at temperatures below 20° C.

Adults of *Laemophloeus pusillus* (Schönh.), the flat grain beetle, are able to withstand temperatures as low as 2° C. for 60 hours (Williams 1954), and *L. ferrugineus* (Steph.), the rusty grain beetle, is reputed to be more resistant to cold than *L. pusillus* or *L. turcicus* (Grouv.) (Cotton 1953). During this survey both *L. ferrugineus* and *L. pusillus* were collected in an unheated mill which had not been in operation for 4 months before the date of collection, February 22, 1955. At the time of collection the temperature was 3° C. It is assumed, however, that the temperature approached or went below that point several times, inasmuch as there were 60 days of "below freezing weather" previous to the date of collection (U. S. Weather Bureau, communication).

Although no specimens of *Tribolium confusum* were

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Table 1.—Incidence of beetles infesting stored products in mills. October, 1954–March, 1955. Buffalo, N. Y.

	Mill Number[a]											Total Mills Infested by Each Species
	A1	A2	B1	B2	C1	C2	D1	E1	E2	E3	E4	
Alphitobius diaperinus (Panz.)						x						1
Attagenus piceus (Oliv.)	x		x			x	x	x	x	x	x	8
Carcinops sp.						x						1
Gnathocerus cornutus (F.)							x					1
Laemophloeus pusillus (Shönh.)	x	x									x	3
Laemophloeus ferrugineus (Steph.)		x				x					x	3
Laemophloeus turcicus (Grouv.)	x											1
Latheticus oryzae (Waterh.)						x			x			2
Oryzaephilus surinamensis (L.)	x	x	x	x		x		x		x	x	8
Palorus ratzeburgi (Wissm.)							x					1
Palorus subdepressus (Woll.)						x						1
Ptinus fur (L.)									x			1
Ptinus hirtellus (Sturm.)	x											1
Rhyzopertha dominica (F.)			x									1
Sitophilus granarius (L.)	x	x			x	x						4
Sitophilus oryza (L.)		x				x						2
Stegobium paniceum (L.)				x					x			2
Tenebrio molitor L.	x							x		x	x	4
Tenebrio obscurus F.	x											1
Tenebroides mauritanicus (L.)	x	x				x		x	x		x	6
Typhaea stercorea (L.)						x						1
Tribolium castaneum (Hbst.)		x				x						2
Tribolium confusum Duv.		x				x	x		x	x		5

[a] A, Retail suburban feed mill; B, Retail urban feed mill; C, Flour mill; D, Feed and flour mill; E, Feed mill.

taken at less than 11° C., Nagel (1934) found that exposure of 24 hours at 21.2° F. was necessary to kill all stages of this insect. Shepard (1939) stated that *T. confusum* died in 3 weeks or less at 45° F.

The possibility of exterminating flour mill insects by freezing was proposed by Cotton & Frankenfeld (1942). A mill was exposed for 48 hours to prevailing outdoor temperatures of −5° F. While the coldest temperature within the mill reached 2° F., the temperature inside a bag of sweepings went down to only 24° F. and other accumulations of grain dust were similarly higher. At the end of this exposure some *Tribolium*, *Tenebroides*, and *Laemophloeus* were still alive.

Of great significance was the almost total absence of insects in mill C1. Although samples were taken from all places which might have been expected to contain insects, only one specimen was found. It is the policy of this mill to clean, completely, every 2 weeks each crack and crevice where refuse accumulates. No dust or other material is allowed to stand longer than 2 weeks. Where chemical sprays are not used, forced air alone is employed to remove all dust and accumulations.

In the light of these findings, the elimination of all possible breeding places is a very important part of any successful insect control program. It is evident that insect control must be continued throughout the entire year, even if the number of insects are small in winter, for it is the expansion of these residual breeding stocks carried over in favorable environments within the mill that helps to account for the increase in mill insects during the summer. This conclusion is supported by Cotton *et al.* (1953) who reported that outbreaks of stored-grain insects originate chiefly from small indigenous insect populations that maintain themselves under adverse conditions or in local favorable situations until conditions are favorable for expansion.

SUMMARY.—A winter survey of 11 feed and flour mills at Buffalo, New York, showed significant residual insect populations. Twenty-three species of coleoptera were collected. *Attagenus piceus*, *Oryzaephilus surinamensis*, *Tenebroides mauritanicus* and *Tribolium confusum* were the most prevalent.

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Effectiveness of Synergized Allethrin and Pyrethrum Sprays Against Mosquitoes[1]

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Most of the work with synergists for pyrethrum and allethrin has been done with the house fly as the test insect. However, there are a few reports of such tests with adult mosquitoes, including those by David & Bracey (1944), McGovran & Fales (1944), Schroeder *et al.* (1948), Waterhouse (1947), Waterhouse & Atherton (1947), Rajindar Pal *et al.* (1952), and Fales *et al.* (1954).

At Beltsville, Md., it has been customary to test insecticidal materials on adult mosquitoes as well as on house flies whenever possible. In most of this mosquito work free-flying insects have been used. Of particular interest has been the trial of materials as synergists for pyrethrum. Since allethrin was found to be less effective than pyrethrum against mosquitoes (Fales *et al.* 1951, 1952), tests have been continued with allethrin plus some of the pyrethrum synergists. This paper reports tests with deodorized-kerosene sprays containing piperonyl butoxide, sulfoxide, MGK-264, or propyl isome as a synergist for allethrin and pyrethrins. The yellow-fever mosquito, *Aedes aegypti* (L.), and the common malaria mosquito, *Anopheles quadrimaculatus* Say, both laboratory-reared, were used as test insects.

As the work on mosquitoes has progressed, the spray dosage has been standardized at 3 ml. per Peet-Grady chamber, or 13.9 ml. per 1000 cubic feet. Since *Aedes* mosquitoes are more susceptible to pyrethrum and allethrin than *Anopheles* mosquitoes, different exposure periods were used. Mortality counts were made on the day following exposure, at which time the insects were sexed.

The insecticides alone were used at two concentrations as checks on the effectiveness of the synergists. These concentrations varied with the material and the insect used. They were determined by previous work and were chosen to give a high and a low point sufficiently separated to show differences between the synergists but still within the range of middle kill. These checks were compared with formulations containing the pyrethrins or allethrin at the concentration of the lower check plus the synergist at six times that concentration. A material giving results similar to those obtained with the higher concentration of the check was judged a good synergist.

TESTS WITH *Aedes Aegypti.*—These mosquitoes were exposed for 5 minutes, but were not collected until the chamber had been vented for 10 minutes. The results of these tests are shown in table 1.

Allethrin alone was one-eighth as effective as the pyrethrins alone, as in previous results with mosquitoes (Fales 1952).

In the pyrethrum sprays piperonyl butoxide caused the greatest synergism against both males and females. Sulfoxide and propyl isome also showed synergism, but MGK-264 little. Preliminary tests with the synergists alone at the same concentration as used in the tests with pyrethrins showed no knockdown or kill except 2 per cent kill of the females with MGK-264. Knockdown was high with each of the pyrethrins-synergist mixtures and with the pyrethrins alone.

With allethrin the synergism was less pronounced than with pyrethrins. Piperonyl butoxide, sulfoxide, and propyl isome caused similar synergism. MGK-264 caused no synergism. Each of the materials gave high knockdown.

TESTS WITH *Anopheles quadrimaculatus.*—A 10-minute exposure was used and the insects were collected immediately after the exposure. The results of these tests are shown in table 2.

With the pyrethrins piperonyl butoxide, sulfoxide, and propyl isome caused good synergism. MGK-264 was the least effective. Preliminary tests with the synergists alone at the same concentrations as used in the tests with pyrethrins showed no knockdown or kill except 6 per cent kill of the females with MGK-264. Knockdown was high with each of the pyrethrins-synergist mixtures and with the pyrethrins alone.

With allethrin the synergism was less pronounced than with pyrethrins. Piperonyl butoxide, sulfoxide, and MGK-264 gave equal performance against females, but propyl isome was even more effective. In preliminary tests with the synergists alone at 2.4 mg. per milliliter, piperonyl butoxide killed 2 per cent, sulfoxide 5 per cent, and MGK-264 12 per cent of the females, but propyl isome caused no mortality. All the materials except propyl isome gave slight knockdown. Knockdown was high with all the allethrin materials.

DISCUSSION.—With all the sprays the mortalities of males were higher than those of females, and those of *Aedes* showed the same relationships between the synergists; however, the mortalities of *Anopheles* males were almost complete.

In previous tests piperonyl butoxide and sulfoxide gave equal performance in pyrethrum sprays against *Anopheles quadrimaculatus,* but against the northern house mosquito, *Culex pipiens* L., sulfoxide was less effective (Fales *et al.* 1954). In tests with free-flying *Culex fatigans* Wiedemann, Rajindar Pal *et al.* (1952) found synergism with a spray containing pyrethrins and pieronyl butoxide and with one containing allethrin and propyl isome. Ginsburg (1951), in tests against *Aedes aegypti,* found pyrethrins to

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Table 1.—Effectiveness of synergized pyrethrum and allethrin sprays against adult *Aedes aegypti* mosquitoes.

SYNERGIST	PER CENT KILL IN 1 DAY	
	Males	Females
Pyrethrins 0.033 mg.+synergist 0.198 mg. per ml. (6 tests)		
Piperonyl butoxide	85	77
Sulfoxide	77	67
MGK-264	66	45
Propyl isome	66	65
No synergist, pyrethrins		
0.033 mg. per ml.	68	39
0.066	89	66
Allethrin 0.264 mg.+synergist 1.584 mg. per ml. (5 tests)		
Piperonyl butoxide	75	63
Sulfoxide	75	59
MGK-264	55	44
Propyl isome	68	61
No synergist, allethrin		
0.264 mg. per ml.	61	47
0.528	83	71

Table 2.—Effectiveness of synergized pyrethrum and allethrin sprays against adult *Anopheles quadrimaculatus* mosquitoes.

SYNERGIST	PER CENT KILL IN 1 DAY	
	Males	Females
Pyrethrins 0.2 mg.+synergist 1.2 mg. per ml. (7 tests)		
Piperonyl butoxide	99	89
Sulfoxide	99	92
MGK-264	92	70
Propyl isome	97	84
No synergist, pyrethrins		
0.2 mg. per ml.	91	60
0.6	98	83
Allethrin 0.4 mg.+synergist 2.4 mg. per ml. (6 tests)		
Piperonyl butoxide	96	78
Sulfoxide	99	78
MGK-264	96	77
Propyl isome	99	84
No synergist, allethrin		
0.4 mg. per ml.	97	65
1.2	99	84

be 2.5 and 4 times as toxic as allethrin to larvae and pupae, respectively. He also found that the toxicity of allethrin to *Aedes* larvae was not materially increased by the addition of piperonyl butoxide, sulfoxide, or propyl isome in a 1:5 ratio. Gersdorff *et al.* (1951) obtained the following descending order of effectiveness against house flies: pyrethrins plus piperonyl butoxide, allethrin plus piperonyl butoxide, allethrin plus propyl isome, pyrethrins plus propyl isome, allethrin plus MGK-264, and pyrethrins plus MGK-264. As shown in table 2, the authors also found that MGK-264 synergized allethrin better than pyrethrins when used against *Anopheles*. However, against *Aedes* it showed no synergism with allethrin. It is possible that the increased effectiveness of allethrin with MGK-264 was not a synergistic action but an additive effect.

In spray tests against house flies Jones *et al.* (1950) found that, in general, combinations of allethrin with a synergist were less effective than similar combinations of pyrethrins. In low-pressure aerosol tests against house flies Schroeder *et al.* (1950) found that allethrin with piperonyl butoxide was about one-half as effective in knockdown and in mortality as similar combinations of pyrethrins. In high-pressure aerosol tests Fales *et al.* (1951) obtained greater mortality of house flies with propyl isome than with piperonyl butoxide when used with allethrin. Against male American cockroaches in powder tests Piquett (1949) found that piperonyl butoxide and propyl isome equally synergized allethrin. In formulating sprays for use against flying insects it seems advisable to consider the results of tests against mosquitoes as well as house flies, since some materials commonly used cause little or no synergism against mosquitoes.

SUMMARY.—In spray tests against the mosquitoes *Aedes aegypti* (L.) and *Anopheles quadrimaculatus* Say, piperonyl butoxide, sulfoxide, propyl isome, and MGK-264 were compared as synergists for both allethrin and pyrethrins.

Against *Aedes* piperonyl butoxide was the most effective when used with pyrethrins, but sulfoxide and propyl isome also gave good synergism. MGK-264 caused no synergism. With allethrin the degree of synergism was not as great as with pyrethrins. MGK-264 again caused no synergism, but the others were nearly equal.

Against *Anopheles* when the synergists were used with pyrethrins the results were similar to those against *Aedes*. With allethrin, however, MGK-264 was equal to piperonyl butoxide and sulfoxide and propyl isome was slightly more effective. Three of the synergists were ineffective when tried alone whereas MGK-264 showed slight toxicity.

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Further Studies on the Attraction of Pink Bollworm Moths to Ultraviolet and Visible Radiation[1]

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In the early investigations of response of the pink bollworm moth, *Pectinophora gossypiella* (Saund.) to lights, it was shown that this moth is not attracted to ordinary incandescent lamps. Recently however, it was found to be strongly attracted to black-light fluorescent and mercury-vapor lamps (Glick & Hollingsworth 1954). This finding stimulated light-trap research, and both field and laboratory experiments showed that not only the pink bollworm moth but many other insects were attracted to lamps radiating energy in the ultraviolet and visible regions of the electromagnetic spectra.

In 1953 tests were conducted at College Station, Texas (Glick & Hollingsworth 1955) to determine the response of the pink bollworm moth to several types of lamps. In 1954 the studies were continued at the Pink Bollworm Research Center, Brownsville, Texas. These experiments were conducted to obtain information on (1) the effectiveness of traps with and without a motor-driven suction fan, (2) the attractiveness of argon glow lamps, (3) electrocuter grid traps in reducing infestation, (4) time of night the moths were attracted to the traps, (5) effect of height of traps above the ground on the attraction of moths, (6) directional response of moths to light traps placed with and against the prevailing wind direction, and (7) the use of light traps (detection traps) in determining the presence of moths and their relative abundance.

SUCTION-TYPE TRAPS.—From August 7 to 20 two traps equipped with suction fans and black-light fluorescent lamps were used with and without the fan in operation. The traps were approximately 10 feet apart, and in each trap the fan was operated on alternate nights. Of the total number of pink bollworm moths taken, 76 per cent were collected in the trap where the fan was operating.

To determine if the suction-type light traps had any effect on the degree of infestation in a given area, two such traps were placed 300 feet apart at opposite ends of a 1½-acre plot of cotton, and counts were made at each side of the plot midway between the traps. On June 16, when the traps were installed, less than 1 per cent of the blooms were infested, but by July 21, 84 per cent of the bolls were infested.

In another experiment a suction-type black-light trap was placed at one end of a 100-acre cotton field and operated from June 21 to July 20 to determine if this type of trap would check an existing 12 per cent boll infestation. Counts were made 100, 300, 500, and 700 feet from the trap. By July 20 all bolls throughout the field were infested. It was concluded that a single suction-type trap was not adequate to prevent or check a pink bollworm infestation.

ARGON GLOW LAMPS.—From June 29 to July 16 a trap containing three 2-watt argon bulbs (essentially low-intensity black-light lamps), although much less visible to the human eye than the black-light fluorescent lamp, was highly attractive to this moth, but it attracted a smaller number of other insects.

In traps equipped with argon lamps more female than male moths were caught. Of 567 moths collected while they were in operation, 58 per cent were females. Of 315 moths collected by the Pink Bollworm Control Project in traps containing argon lamps, 75 per cent were females, and in one trap in which 104 moths were taken in one night, 94 per cent were females. In traps fitted with black-light fluorescent or black-light blue fluorescent lamps the males predominated (Glick & Hollingsworth 1955, Pfrimmer *et al.* 1955).

ELECTROCUTER-GRID TRAPS.—An electrocuter-grid trap (Fig. 1) equipped with two black-light fluorescent lamps was tested to determine if it would prevent a pink bollworm infestation. The trap was operated continuously from April 18 to July 15 on a 1/20-acre plot enclosed in a tightly screened cage. On April 18 to 20, 116 pink bollworm moths of both sexes were released in the cage. Blooms did not appear until the last of April. By June 9, 12 per cent of the blooms and 20 per cent of the bolls were infested. On June 23, 48 per cent of the bolls were infested, and by the middle of July, 100 per cent. From June 3 to July 14, 2,163 moths that had been killed by the electrocuter grid were counted. Undoubtedly many more moths had been killed but had not fallen into the collection tray below the grid. These results indicate that a single electrocuter grid trap will not prevent a build-up of infestation in a given area.

TIME OF NIGHT COLLECTED.—A trap was equipped

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[2] In cooperation with the Texas Agricultural Experiment Station. L. R. Crockett, H. T. Wiedmann, and S. O. Smith assisted in these studies.

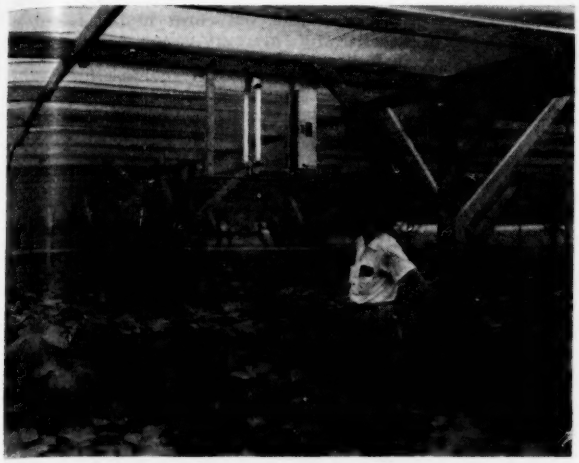

FIG. 1.—Electrocuter grid trap placed in a large screen cage for test on reduction of a pink bollworm infestation.

with a suction fan and four black-light fluorescent lamps, and the moths caught were counted hourly from 8 P.M to 6 A.M. from July 23 to 30. As shown in table 1, 40 per cent of the moths were collected between 2 and 4 A.M. More females than males were caught from 11 P.M. to 1 A.M., but males outnumbered females from 8 to 9 P.M. and from 2 A.M. to daybreak. Lukefahr (unpublished report) found that oviposition was heaviest before midnight. Apparently the moths become more active in the air after oviposition has taken place.

These tests were made during the dark of the moon. Similar ones were not made during moolight nights. However, it is known that moonlight does increase insect activity in general, although Lepidoptera appear to be more active on dark nights (Glick 1939). In the Punjab area of India, Husain *et al.* (1934) found some evidence that greater numbers of pink bollworm moths were attracted on dark than on bright nights. Busk (1917) found these moths to be more active from 6:30 to 8 P.M. in Hawaii. Loftin *et al.* (1921) reported that in Mexico moths were seen in fields from dusk to midnight, when their observations were terminated. Fenton & Glick (unpublished) observed moths active throughout the night in cotton fields at Tlahualilo, Durango, Mexico, in 1928. Husain *et al.* (1934) reported from Punjab that they were attracted to lights in greater numbers from 2 to 6 A.M., and the period from 10 P.M. to 2 A.M. came next. However, in another experiment they showed 6 to 10 P.M. to be the most favorable. An incandescent gas lamp of 200 candlepower was used in the traps in Punjab.

EFFECT OF HEIGHT OF TRAPS.—To determine the relative response of moths to lamps placed at different heights, seven traps were placed on poles at 2-foot intervals from 2 to 14 feet above the ground and 17 feet apart (Fig. 2) at the edge of a cotton field. Each night from July 23 to August 30 the traps were placed at random at the various heights. They were designed in such manner that the lamps radiated their energy toward a 100-acre cottonfield. As shown in table 2, of the total number of moths taken, 39 per cent were at the 2-foot height and 71 per cent at the three lowest levels. The number decreased as the elevation increased, only 6 per cent being captured at 14 feet. These results confirm previous observations that pink bollworm moths normally fly close to the ground. At all heights the males outnumbered the females.

DIRECTIONAL RESPONSE.—To determine whether the pink bollworm moth flies into the traps with or against the prevailing wind, two mercury-vapor traps were mounted side by side, one facing the southeast and the other the northwest (Fig. 3). The prevailing wind during the late spring and summer in the Lower Rio Grande

FIG. 2.—Seven traps containing black-light fluorescent lamps placed on poles at intervals from 2 to 14 feet above the ground.

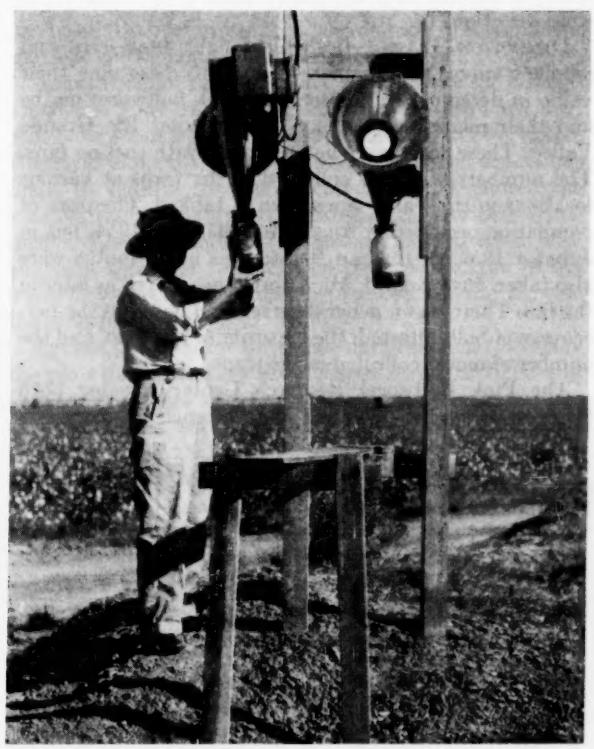

FIG. 3.—Two mercury-vapor traps placed in opposite directions, one facing and the other against the prevailing wind.

Table 1.—Pink bollworm collections in a light trap equipped with a suction fan and four black-light fluorescent lamps, at different times of night, July 23 to 30, 1954, Brownsville, Texas.

Time	Number of Moths	Per Cent of Total	Per Cent Females
7– 8 P.M.	103	1.3	21
8 –9	470	5.9	44
9–10	503	6.3	52
10–11	580	7.3	55
11–12 M.	664	8.3	56
12– 1 A.M.	805	10.1	51
1– 2	934	11.7	44
2– 3	1,584	19.9	16
3– 4	1,634	20.5	14
4– 5	471	5.9	19
5– 5:30	202	2.5	27
5:30– 6	23	0.3	30
Total	7,973	100.0	33

Valley is from the southeast, but there were times when the wind direction changed. For the entire season from May to October, 86 per cent of the moths collected entered the trap facing the northwest. In other words, they apparently flew into the prevailing southeast wind. On the nights when the wind was 6 or more miles per hour few moths were collected. The greatest number were taken when it was 3 miles per hour or less. This had been previously determined at College Station in 1953; when pink bollworm moths were released in a laboratory wind tunnel, they were not able to regulate their flight and respond to the lights with wind currents over 3 miles per hour. There was very little difference in sex response to wind direction.

DETECTION TRAPS.—Both black-light fluorescent and mercury-vapor light traps were used to determine their value in detecting the presence of pink bollworm moths and their relative abundance in the Lower Rio Grande Valley. These traps were not equipped with suction fans. The numbers of moths collected in the traps at various locations in the Valley are shown in table 3. The peak of population occurred in August for 1953 and 1954, but in July for 1952. In the San Benito area a few moths were also taken during April, May, and June, as well as later in the fall. There was a general correlation between the percentage of bolls infested, the maturity of the crop, and the number of moths collected in the traps.

The Pink Bollworm Research Center is using both types of traps for detecting the presence of pink bollworm moths and other insects in many locations in Texas, also in New Mexico, Louisiana, and Mississippi, and the Pink Bollworm Control Project has used them and also some with argon lamps in survey work in cooperation with the States of Arkansas, Oklahoma, Louisiana, Mississippi, and Tennessee. Several of these traps are being operated in cotton-mill and seed-storage houses.

Table 2.—Pink bollworm collections in light traps placed at various heights above the ground, July 23 to August 30, 1954, Los Fresnos, Texas.

Height (Feet)	Number of Moths	Per Cent of Total	Per Cent Females
2	11,748	39	23
4	4,812	16	17
6	4,707	16	20
8	2,375	8	17
10	2,251	8	12
12	2,028	7	14
14	1,776	6	14
Total	29,697	100	19

Table 3.—Numbers of pink bollworm moths taken in black-light fluorescent and mercury-vapor traps in the Lower Rio Grande Valley, July-September, 1954.

Location and Type of Trap	Number of Traps	July	August	September	Total
San Benito:					
Black-light	4	16,675	126,685	11,083	154,443
Mercury-vapor	3	8,857	31,178	1,469	41,504
Bayview:					
Black-light	1	373	2,911	240	3,524
Mercury-vapor	1	8	8	2	18
Los Fresnos:					
Black-light	7	8,634	22,079	134	30,847
Mercury-vapor	2	2,015	3,230	970	6,215
Brownsville:					
Black-light	3	—	15,465	1,344	16,809
Total	21	36,562	201,556	15,242	253,360

Traps with argon lamps collected more pink bollworm moths per volume of total insects and a higher percentage of female moths than those with black-light lamps.

One electrocuter-grid trap in a caged 1/20-acre plot of cotton into which moths were introduced did not control or prevent a build-up to a 100 per cent boll infestation.

The moths responded to black-light lamps in greatest numbers between 2 and 4 A.M.

The height of the traps above the ground affected collections, 39 per cent being caught at the 2-foot height and 71 per cent below 6 feet.

In tests to determine whether the moths flew into or away from the prevailing wind, 86 per cent of those trapped flew into the wind.

In light traps placed at various locations in the Lower Rio Grande Valley to detect the presence of moths and their relative abundance, more moths were taken during August than in July or September. The number of moths taken in the traps was correlated with the infestation in the field and crop maturity.

SUMMARY.—In 1954 field studies of the pink bollworm, *Pectinophora gossypiella* (Saund.), with traps containing lamps radiating in the ultraviolet and visible spectra were continued at Brownsville, Texas.

Of the moths collected in traps equipped with or without a suction fan, 76 per cent were taken in the one with the fan.

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Control of the Clover Root Borer and the Meadow Spittlebug on Red Clover[1,2]

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The clover root borer, *Hylastinus obscurus* (Marsh.), and the meadow spittlebug, *Philaenus leucophthalmus* (L.), are two of the most important insects attacking red clover. The clover root borer, first reported damaging red clover in New York State in 1878 (Riley 1879), now occurs in all areas of northern United States and Canada wherever this crop is extensively grown, and in some years has caused considerable damage. The meadow spittlebug has become increasingly injurious to red clover and other legume forage during the past decade. Serious losses to first-cutting legume hay have been recorded from Illinois and Wisconsin eastward to the Atlantic seaboard. As a result of research carried on by many workers, spraying in early spring with lindane, toxaphene, or methoxychlor is now recommended for control of the spittlebug. The use of insecticides for control of the root borer has not been widely recommended.

Marshall *et al.* (1949) reported that BHC at 1.5 pounds of gamma isomer per acre furnished excellent control of the clover root borer. App & Everly (1950) showed that BHC at 1.25 pounds of gamma per acre, aldrin at 2 pounds, chlordane at 5 pounds, and parathion at 5 pounds gave good control when applied as dilute dusts either in September or in May. In addition, all these insecticides except parathion furnished excellent control of the meadow spittlebug.

From 1949 to 1952 the most promising materials were tested at different dosages in both spring and fall applications. Additional experiments were conducted to compare the effectiveness of sprays and dusts, and also to determine the practicability of utilizing commonly available farm machinery to apply the dusts. Data on the insecticide residues present on clover forage and on the effect of the treatments on the seed yields of red clover were obtained from most of the experiments.

GENERAL PROCEDURE.—Fall applications were made in September and spring applications in May. Most of the treatments were applied to small plots ranging from 18 to 30 feet square. A Latin square or a randomized-block arrangement replicated 4 to 5 times was used.

Most of the dusts contained 1 per cent of the active ingredient. Dust applications to small plots were made with a hand-operated fertilizer distributor that treated a 3-foot strip of forage and to larger plots with a tractor-drawn fertilizer distributor. Wettable-powder sprays were tried in 1950, but in 1951 and 1952 emulsion sprays were used. The sprays were applied to small plots with a hand compressed-air sprayer and to large plots with a power sprayer mounted on a pickup truck. The power sprayer was operated at approximately 50 p.s.i. and delivered about 6 gallons per acre.

The effect of the treatments on the clover root borer was determined in late July or in August of each year by digging and dissecting a sample of roots and recording the numbers of infested roots and borers found. Usually 5 samples of 3 roots were taken in each plot.

The effect of the treatments on the meadow spittlebug was determined by taking samples of the nymphal population in May of each year. To aid in taking the samples a heavy wire rod 36 inches long marked with red paint at five equidistant places was dropped lengthwise at random in the crop and the number of nymphs on the stem nearest to each red mark was recorded. In most of the experiments 5 samples totaling 25 stems were examined in each plot.

FALL APPLICATIONS.—Experiments were conducted with dusts in the fall of 1949 and 1950 and with emulsion sprays in the fall of 1951. The insecticides and the dosages used and their effect on the clover root borer and the meadow spittlebug are summarized in table 1.

Root dissections made in 1950 showed that the dosages applied in 1949 were too light for good control of clover root borers, but that aldrin and technical BHC were more effective than the other insecticides. Root dissections made in 1951 in the plots treated in 1950 showed the greatest reductions from the aldrin and chlordane treatments. Root dissections in 1952 in plots treated with emulsion sprays in 1951 showed that none of the sprays were very effective but that heptachlor and aldrin were the most promising.

Against the meadow spittlebug data taken in 1950 from the plots dusted in 1949 showed that technical BHC and aldrin were most effective. Chlordane was much less effective in this test. Data taken in 1951 in plots dusted in 1950 showed that all the dusts except chlordane furnished good control. Data taken in 1952 in plots sprayed in 1951 showed that heptachlor and technical BHC were more effective than chlordane or aldrin.

SPRING APPLICATIONS.—With most of the insecticides used in the fall comparable tests were made in the spring. Dusts were applied in 1950 and 1951 and emulsion sprays in 1952. Table 2 lists the insecticides, the dosages used,

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[2] The research reported in this paper was conducted at the Legume Seed Research Laboratory, Columbus, Ohio, maintained cooperatively from 1948 to 1953 by the U. S. Department of Agriculture and the Ohio State University. Ralph H. Davidson, Department of Zoology and Entomology, Ohio State University, made valuable suggestions during the conduct of these experiments.

Table 1.—Effect of insecticides applied in the fall on populations of the clover root borer and the meadow spittlebug the following year. 1949–51.

Insecticide	Active Ingredient per Acre	Roots Infested	Average Borers per 15 Roots	Decrease or Increase in Borers as Compared with Check	Reduction in Spittlebugs
	Pounds	*Per Cent*	*Number*	*Per Cent*	*Per Cent*
		Dusts applied in 1949			
None	—	37.3	10.2	—	—
Aldrin	1.50	13.3	4.0	−61	90
Technical BHC (12–14%)[a]	1	17.3	6.4	−37	100
Chlordane	3	37.3	13.6	+33	50
Purified BHC (98%)[a]	1	34.7	17.2	+69	80
L.S.D. 5%	—	—	None		
		Dusts applied in 1950			
None	—	56.0	52.0	—	—
Aldrin	1.75	5.3	1.0	−98	98
Chlordane	4	9.3	5.0	−90	76
Technical BHC (12–14%)[a]	1.25	26.7	14.8	−72	99
Lindane	1.25	34.7	22.8	−56	93
L.S.D. 5%	—	—	20.2		
1%	—	—	28.3		
		Emulsion sprays applied in 1951			
None	—	86.7	95.2	—	—
Heptachlor	1.75	58.7	52.8	−44	91
Aldrin	1.75	66.7	64.6	−32	71
Technical BHC (12–14%)[a]	1.75	86.7	93.8	− 1	89
Chlordane	4	86.7	108.2	+13	81
L.S.D. 5%	—	—	35.2		
1%	—	—	49.4		

[a] Gamma isomer.

Table 2.—Effect of insecticides applied in the spring on the population of the clover root borer and the meadow spittlebug. 1950–52.

Insecticide	Active Ingredient per Acre	Roots Infested	Average Borers per 15 Roots	Decrease or Increase in Borers as Compared with Check	Reduction in Spittlebugs
	Pounds	*Per Cent*	*Number*	*Per Cent*	*Per Cent*
		Dusts applied in 1950			
None	—	18.7	6.2	—	—
Chlordane	5	1.3	0.8	−87	100
	3	8.0	1.8	−71	100
Parathion	5	12.0	3.2	−48	100
Lindane	1	13.3	4.4	−29	100
	.75	16.0	6.0	− 3	100
Technical BHC (12–14%)[a]	.75	9.3	4.8	−23	100
	1	6.7	5.4	−13	100
Aldrin	1.25	13.3	5.8	− 6	100
	.50	21.3	9.8	+58	100
L.S.D.	—	—	None		
		Dusts applied in 1951			
None	—	48.4	59.0	—	—
Heptachlor	1.75	0.0	0.0	−100	100
	1.50	8.3	1.5	−97	100
Aldrin	1.75	1.7	0.8	−99	100
	1.50	13.3	4.8	−92	100
Perthane	4	25.0	19.8	−66	100
L.S.D. 5%	—	—	29.8		
1%	—	—	41.3		
		Emulsion sprays applied in 1952			
None	—	77.3	77.6	—	—
Heptachlor	1.50	52.0	43.4	−44	100
	1	58.7	46.0	−41	98
Dieldrin	.75	62.7	59.6	−23	100
Isodrin	.75	82.7	97.8	+26	98
L.S.D. 5%	—	—	37.1		
1%	—	—	52.0		

[a] Gamma isomer.

and their effect on the clover root borer and the spittlebug.

Roots dissected from plots dusted in 1950 showed that most of the dosages were too light for root borer control, but chlordane was the most effective. Roots dissected from plots dusted in 1951 indicated that aldrin and heptachlor gave very good control of the root borer. These insecticides were somewhat more effective at $1\frac{3}{4}$ pounds per acre than at $1\frac{1}{2}$ pounds. Perthane was considerably less effective. Dissections from plots sprayed in 1952 showed that none of the emulsion sprays furnished good control of the clover root borer. Heptachlor at both dosages showed more promise than the other insecticides.

All the insecticides used in the spring applications gave excellent control of the spittlebug.

Comparison of Sprays and Dusts.—The results of the experiments to compare the results obtained with sprays and dusts are given in table 3.

Data from 15 roots per plot dissected from plots treated in 1950 showed that all the treatments significantly reduced the root borer population below that in untreated plots but that there were no significant differences between wettable-powder sprays and dusts.

In May 1951, plots of Kenland red clover 60×200 feet in randomized blocks replicated four times were treated with dusts and emulsion sprays of aldrin and BHC applied with power equipment. The effect on the clover root borer was determined by digging 18 roots per plot. BHC in a dust provided good control of the root borer but was ineffective when applied in an emulsion spray. Aldrin was equally effective when applied in a dust or in an emulsion spray.

In May 1952, plots 63×350 feet were sprayed with heptachlor, aldrin, and BHC with power equipment.

Table 3.—Comparative effectiveness of sprays and dusts against the clover root borer and the meadow spittlebug. 1950–52.

Insecticide	Active Ingredient per Acre[a]	Roots Infested	Average Borers per 15 Roots	Decrease in Borers Over Check	Reduction in Spittlebugs
	Pounds	*Per Cent*	*Number*	*Per Cent*	*Per Cent*
		Treated in 1950			
None	—	32.0	12.6	—	—
BHC, spray (WP)	1	17.3	3.1	75	100
dust	1	8.0	1.4	89	100
Aldrin spray (WP)	1	14.7	4.6	64	100
dust	1	10.7	5.4	57	100
Chlordane spray (WP)	5	8.0	2.1	83	98
dust	5	16.0	3.4	73	100
L.S.D. 5%			6.5		
1%			8.7		
		Treated in 1951			
None	—	37.5	28.0[b]	—	—
BHC, dust	2	9.7	3.0	89	100
spray (Em)	2	36.1	24.8	12	100
Aldrin dust	2	7.0	2.0	93	100
spray (Em)	2	8.4	1.5	95	100
L.S.D. 5%			19.3		
1%			27.1		
		Treated in 1952			
Sprays:					
None	—	81.7	87.2	—	—
Heptachlor (Em)	1.75	53.3	34.5	60	100
Aldrin (Em)	1.75	73.3	76.0	13	98
BHC, (Em)	1.75	63.3	61.0	30	100
L.S.D. 5%			None		
1%					
Dusts:					
None	—	85.0	120.5	—	—
Heptachlor	1.75	13.3	0.8	99	100
Aldrin	1.75	35.0	20.0	83	100
BHC	1.75	36.7	23.0	81	100
L.S.D. 5%			38.2		
1%			54.9		

[a] BHC expressed in pounds of gamma isomer.
[b] Data based on 18 roots.

Heptachlor was the most promising insecticide for root borer control, although none of the sprays were very effective. The same insecticides in the form of dusts were applied in the same field at the same dosages. The plots were 30×30 feet and arranged in a Latin square. Samples of dissected roots showed heptachlor to be the most effective against the root borer, followed by aldrin, and BHC.

The meadow spittlebug was controlled with all three of these insecticides, samples of stems showing reductions of over 98 per cent whether the material was applied as a spray or dust.

APPLICATION OF DUSTS WITH COMMONLY AVAILABLE FARM MACHINERY.—In May 1950 two experiments were conducted in which commonly available farm machinery was used to apply the dusts.

BHC at 0.84 pound of gamma isomer, aldrin at 0.75 pound, and chlordane at 3.23 pounds per acre were applied to 1-acre plots with a grain drill the tubes of which had been removed from the shoes and allowed to hang loose. Good distribution of the dust was observed. Twenty samples of 5 roots dissected from each plot showed that BHC reduced the root borer population 77 per cent, aldrin 68 per cent, and chlordane 64 per cent when compared with the untreated plot. Examination of 50 samples of 5 stems from each plot showed that the spittlebug was practically eliminated by all these dusts.

In the second experiment a fertilizer distributor that covered a 10-foot strip of forage was used to apply aldrin at 1.82 pounds and BHC at 1.47 pounds of gamma isomer per acre. Dissection of 100 roots per plot showed that the plots treated with aldrin averaged 98 per cent and those treated with BHC 82 per cent fewer root borers than untreated plots. No spittlebug nymphs were found in 25 samples of 5 stems examined in each of these plots.

INSECTICIDE RESIDUES.—In June of 1951 and 1952 samples of clover forage were taken for determinations of the residues present on the hay at harvesttime. In each of the plots sampled two 1-kg. lots of green forage were cut and air-dried, and submitted for chemical analyses. The results are given in table 4. In most of the experiments the residues were higher after the spring treatments than after those in the fall. More information is needed before definite conclusions regarding residues can be reached.

EFFECT OF TREATMENTS ON SEED YIELDS OF RED CLOVER.—In each of these experiments an attempt was made to determine the effect of the treatments on the seed yields. Whenever practical ¼-square-yard samples of seed were harvested and threshed, but in certain experiments the clover stands were so poor and pollination so inadequate that this was not feasible. In these cases a sample of flower heads was dissected to determine the number of seeds per head.

None of the treatments significantly increased the yield of seed or the number of seeds per head. However, in some of the experiments plots treated with heptachlor, aldrin, or BHC, showing the best control of the root borer, averaged more seeds per head than untreated plots.

DISCUSSION.—These experiments show that the clover root borer can be controlled with dusts of several insecticides. The most promising are heptachlor at 1½ to 1¾ pounds, aldrin at 1¾ to 2 pounds, and BHC at 1½ to 2 pounds of gamma isomer per acre. Applications made either in September or in May are effective. In a single experiment wettable-powder sprays of aldrin and BHC were as effective as dusts of these insecticides. Emulsion sprays applied at the rate of 6 gallons of spray per acre were generally unsatisfactory. Increasing the amount of water per acre might give better results with emulsion sprays.

Nymphs of the meadow spittlebug were adequately controlled with aldrin, heptachlor, or BHC applied in September or in May. Dusts and wettable-powder and emulsion sprays all gave excellent results against spittlebugs. Dusts of any of these materials might therefore be used to control both the spittlebug and the root borer.

Red clover is normally limited to one crop year. Observations made in several of the fields indicated that controlling the root borer did not prolong the life of the clover. The importance of clover root diseases and other factors, as well as injurious insects, in the failure of stands after one crop year needs to be determined.

SUMMARY.—From September 1949 through May 1952 several experiments with insecticides for control of the clover root borer, *Hylastinus obscurus* (Marsh.), were conducted in Ohio. The effect of the treatments on

Table 4.—Insecticide residues found on red clover hay following treatments for control of the clover root borer and the meadow spittlebug. 1950–52.

INSECTICIDE	ACTIVE INGREDIENT PER ACRE[a]	RESIDUE (CORRECTED)
	Pounds	*P.p.m.*
Treated in September 1950, harvested in June 1951		
Dusts:		
Aldrin	1.75	0.36
BHC	1.75	1.56
Chlordane	4	1.20
Lindane	1.25	.20
Treated in September 1951, harvested in June 1952		
Emulsion sprays:		
Aldrin	1.75	0.14
BHC	1.75	8.80
Chlordane	4	21.40
Heptachlor	1.75	0
Treated in May and harvested in June 1951		
Dusts:		
Aldrin	1.75	5.65
BHC	2	.98
Heptachlor	1.75	2.02
Perthane	4	.32
Treated in May, harvested in June 1952		
Dusts:		
Aldrin	1.75	4.25
BHC	1.75	10.12
Heptachlor	1.75	12.79
Emulsion sprays:		
Aldrin	1.75	7.18
BHC	1.75	6.41
Dieldrin	.75	2.39
Heptachlor	1.50	6.70
	1	7.00
	1.75	5.09
Isodrin	.75	1.35

[a] BHC expressed in pounds of gamma isomer.

nymphs of the meadow spittlebug, *Philaenus leucophthalmus* (L.), to determine whether both of these important pests could be controlled with a single treatment was also recorded. Of 10 insecticides that were tested, aldrin, BHC, and heptachlor were the most promising.

Heptachlor dust as a spring treatment at 1½ to 1¾ pounds of the active ingredient per acre reduced the clover root borer population 97 to 100 per cent. Emulsion sprays of this material at comparable dosages did not give adequate control.

Fall treatments with dusts of aldrin at 1½ to 1¾ pounds per acre reduced the number of clover root borers 61 to 98 per cent, and comparable spring treatments 83 to 99 per cent. Lower dosages were not satisfactory. In one experiment a wettable-powder spray and a dust at 1 pound per acre were equally effective. With one exception, emulsion sprays of this insecticide were unsatisfactory.

Fall applications of BHC at 1 and 1¼ pounds of gamma isomer per acre reduced the root borer population 37 and 72 per cent, respectively, and spring applications of ¾ to 2 pounds from 13 to 89 per cent. Fair control was obtained with a wettable-powder spray in May 1950, but poor results were obtained with emulsion sprays in May of 1951 and 1952.

Dusts of heptachlor, aldrin, and BHC, which were most promising for clover root borer control, also controlled nymphs of the meadow spittlebug. Most of the other insecticides tested gave good to excellent results against the spittlebug. Dusts, wettable-powder, and emulsion sprays were all effective against the spittlebug.

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Effect of Systemic Insecticides on Green Peach Aphid Populations on Potatoes[1]

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Large populations of the green peach aphid, *Myzus persicae* (Sulz.), may develop and cause damage to potatoes in the Yakima Valley of Washington, but the aphid is of most importance as a vector of the leaf-roll virus disease. Experiments showed that the spread of the disease could be retarded appreciably when the aphids were controlled with insecticides (Gibson *et al.* 1951, Klostermeyer 1953). Either four or five applications of a dust mixture of DDT 5 per cent, parathion 0.5 per cent and sulfur 50 per cent gave good control of the aphid and the disease for several years, but recently the aphid has become increasingly difficult to kill.

MATERIALS AND METHODS.—In 1954, various systemic insecticides applied as sprays were tested on potatoes to determine whether these would provide greater initial kill of the aphid and prevent reinfestation for greater periods than previously tested materials. In these tests, three applications each of seven treatments consisting of 3, 6, and 12 ounces of demeton per acre-application, 16 and 32 ounces of schradan, 12 ounces of parathion, 4 ounces of Isolan per acre-application, and an untreated control were evaluated at the State College of Washinton, Irrigation Experiment Station, Prosser, Wash. Emulsifiable concentrates of each material were used. The schradan formulation contained 4 pounds and each of the other insecticide formulations contained 2 pounds of active ingredient per gallon.

The Russet Burbank potatoes used for these tests were planted May 19. The treatments were applied to plots 7 rows wide and 65 feet long arranged in a randomized block design of 5 replications. A Slosser-type sprayer with four nozzles per row was used in applying 25 gallons of spray per acre at 75 pounds pressure. The potato plants were about 6 inches high when the first application was made on June 16. The second application was made on July 12 and a third on July 25, but no additional applications were made because of the plant damage caused by the spray equipment.

Results were evaluated by counting the aphids present on 50 compound leaves from each plot at intervals during the season. The leaf samples were collected just prior to treatment, 3 and 7 days after treatment and at weekly intervals thereafter. Since the numbers of winged aphids were too small to evaluate, the effectiveness of the insecticides was determined from the numbers of wingless forms. For the analysis of variance the numbers (n) of wingless aphids per 50 compound leaves were converted to the $\sqrt{n+0.5}$. The multiple range test proposed by Duncan (1953) was used to determine significant differences between treatments.

After the potatoes were harvested on September 15, 50 marketable size tubers were examined for net necrosis, the tuber symptom of potato leaf roll. An additional sample was examined in April, 1955. Other samples were used for chemical analysis of residues of demeton and schradan. Samples of the 3- and 12-ounce rates of demeton and the 32-ounce rate of schradan were used for animal feeding trials.

RESULTS.—The aphid population developed slowly in

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[2] E. W. Davis, Entomology Research Branch, U.S.D.A., who died October 24, 1954, assisted with the population counts. W. E. Westlake, Entomology Research Branch, U.S.D.A., supervised the analysis of the insecticide residues.

Table 1.—Effect of systemic insecticides on aphid populations on potatoes following second application July 15, 1954, Prosser, Washington.

TREATMENT (OZ. PER A.)	DATE OF SAMPLING (JULY)			
	12	15	19	26
	$\sqrt{\text{No.}}+0.5$ of aphids per 50 leaves			
Demeton, 3	2.68	2.35[ab]	3.90[cd]	6.11[abd]
Demeton, 6	2.61	1.88[ab]	2.89[cde]	5.69[bd]
Demeton, 12	2.80	2.08[ab]	2.69[cde]	4.78[cd]
Schradan, 16	2.37	1.32[ab]	2.12[de]	4.62[cd]
Schradan, 32	2.55	1.12[b]	1.43[e]	2.75[c]
Parathion, 12	2.54	1.16[b]	4.76[bc]	7.39[ab]
Isolan, 4	2.25	2.71[a]	6.29[ab]	7.62[ab]
Check	2.60	3.80[a]	7.55[a]	9.41[a]
SE of Mean	—	0.343	0.506	0.587

[a] For each date of sampling, any two treatments not having the same superscript are significantly different at the 1 per cent level. Any two treatments having the same superscript are not significantly different. For example, in the July 19 data, the check (7.55[a]) is significantly different from each treatment except Isolan (6.29[ab]). Similarly, 32 oz. of schradan treatment (1.43[e]) is significantly different from 3 oz. demeton, parathion, Isolan or the check but is not different from any of the other treatments.

June and the first application of the insecticide sprays appeared to have little effect. However, after mid-July large flights of the green peach aphid occurred over the field and the crop was subject to daily reinfestation by winged aphids with a resultant increase in wingless aphids. The effects of the insecticides on the population development after the second insecticide application are indicated in table 1. Following the July 12 application, it was apparent that the 12 ounces of parathion and the 2 pounds of schradan were responsible for the sharp reductions in the populations and the 12 ounces of demeton and the 4 ounces of Isolan also caused population reductions. However, within a week the populations in the Isolan-treated plots again equalled those of the untreated check plots and the aphids had increased nearly as rapidly in the parathion plots. The aphids increased slowly in the schradan- and demeton-treated plots.

Although the aphid population apparently declined in the untreated check plots within 3 days after the application on July 26, all of the treatments caused significant reductions in the numbers of aphids at this time (Table 2). For this application, the dosage of Isolan was increased from 4 to 8 ounces per acre. This improved the initial kill but the residual action was not prolonged and after 2 weeks the populations again exceeded the untreated checks. Populations affected by the parathion treatment followed a similar course. Although the 32-ounce schradan treatment resulted in the lowest aphid population on each date of sampling, there was no significant difference between that treatment and the highest rate of demeton. The lower aphid populations in the 32-ounce schradan treatment were significantly different from the populations in the 16-ounce schradan treatment only on July 29 and on August 2.

Table 2.—Effect of systemic insecticides on aphid populations on potatoes following third application July 25, 1954.

TREATMENT (OZ. PER A.)	DATE OF SAMPLING				SEASONAL MEAN	
	JULY 29	AUGUST 2	AUGUST 9	AUGUST 23	$\sqrt{n+0.5}$	n
	$\sqrt{\text{No.}+0.5}$ of aphids per 50 leaves					
Demeton, 3	3.16[b]	4.26[bc]	7.25[bc]	9.31[b]	4.88	24
Demeton, 6	2.12[bcd]	2.76[cd]	5.07[cd]	7.63[bc]	3.83	15
Demeton, 12	1.78[bcd]	2.77[cd]	3.95[d]	7.64[bc]	3.56	13
Schradan, 16	2.42[bc]	3.41[bc]	5.87[cd]	8.22[bc]	3.78	14
Schradan, 32	1.25[d]	1.55[d]	3.70[d]	7.05[c]	2.68	7
Parathion, 12	1.64[cd]	4.66[b]	10.89[a]	12.21[a]	5.66	32
Isolan, 8	2.31[bc]	3.71[bc]	10.21[a]	8.53[bc]	5.45	30
Check	8.09[a]	10.26[a]	9.23[ab]	8.78[bc]	7.47	56
SE of Mean	0.246	0.419	0.552	0.450	—	—

[a] For explanation of superscripts see table 1.

Table 3.—Net necrosis of potato tubers compared with numbers of aphids, Prosser, Washington.

TREATMENT (OZ. PER A.)	PERCENTAGE OF TUBERS WITH NET NECROSIS		NO. OF APHIDS ON 3,500 LEAVES EXAMINED DURING SEASON	
	Sept. 1954	Apr. 1955	Winged	Wingless
Demeton, 3	5.2	12.1	117	1856
Demeton, 6	9.2	11.0	118	1462
Demeton, 12	8.8	14.0	109	1156
Schradan, 16	6.8	12.7	99	1422
Schradan, 32	8.4	14.6	86	870
Parathion, 12	9.2	11.9	139	3171
Isolan, 4 and 8	9.2	12.5	126	2391
Check	11.6	14.8	125	3229

Since demeton had been accepted for national registration for use on potatoes at 3 ounces per acre early in the 1954 season, this dosage was tested along with the higher dosages which were believed might be necessary for the control of the green peach aphid in this area. The 3-ounce rate of demeton treatment had a consistently higher aphid population than the 6- and 12-ounce rates. However, the differences were not significant except on August 9 when the 3-ounce rate of demeton differed from the 12-ounce rate but not from the 6-ounce rate.

During the latter part of August many of the potato plants in the field exhibited symptoms of leaf-roll disease. Early season infection of the Russet Burbank variety results in the development of net necrosis, a dark brown discoloration of the phloem of the tubers. These symptoms are indications of the spread of the disease and, more important, may cause the crop to be rejected for market, if excessive. Appreciable numbers of tubers from each treatment examined shortly after harvest contained net necrosis (Table 3), but the differences between treatments or between treatments and the untreated check were not significant. Net necrosis usually increases in storage. An additional sample examined in April, 1955 had a greater amount of net necrosis but differences among treatments were not significant. However, it is evident that the winged aphids were not killed rapidly enough or in sufficient numbers to prevent the spread of the disease.

Tuber samples were analyzed for translocated residues of demeton and schradan. The results of these analyses are shown in table 4. Small amounts of demeton and schradan were translocated to the tubers. Recovery of known amounts of schradan added to check tubers averaged 60 per cent. Demeton was determined by the method of Hensel *et al.* (1954) and schradan analyses were made by the method of Hall *et al.* (1951).

Potatoes from the 3- and 12-ounce rates of demeton and

Table 4.—Residues of demeton and schradan found in potato tubers, 1954, Prosser, Washington.

Treatment (Oz. per A.)	Residues of Insecticide (p.p.m.) Sample I	II	III	IV	V	Mean	Corrected for Check
Demeton, 3	0.27	0.18	0.22	0.19	0.18	0.21	
Demeton, 6	.50	.41	.33	.29	.37	.38	
Demeton, 12	.61	.34	.09	.24	.34	.31	
Schradan, 16	.28	.38	.46	.43	.22	.35	0.26[a]
Schradan, 32	.54	.68	.93	.51	.74	.68	.59[a]
Check	—	.07	.08	.09	.11	.09	—

[a] Recovery of known amounts of schradan averaged 60 per cent. These figures have not been adjusted for per cent recovery.

the 32-ounce rate of schradan treatment were sent to the Technical Development Laboratories, Communicable Disease Center, U. S. Public Health Service, Savannah, Georgia, where they were fed for 30 days to rats and monkeys as the major item of their diet. No ill effects were noted on either species.[3] The animals gained weight and there was no apparent adverse effect on the cholinesterase levels of the blood.

Discussion.—Demeton and schradan apparently were less effective in 1954 than these materials were in tests made in 1951 (Klostermeyer 1953). However, the aphid population level was greater in 1954 and reinfestation occurred over a longer period of time. Nevertheless, there is an indication of reduced effectiveness of systemic, phosphate insecticides possibly related to the decline in effectiveness of parathion observed during the same period. In addition, the 1951 tests showed a slight but significant decrease in net necrosis as a result of the insecticide treatment. This did not occur in the present tests, probably because of the generally higher population level of aphids.

The 3-ounce rate of demeton which is presently registered for use on potatoes was only slightly more effective than 12 ounces of parathion per acre for the control of the green peach aphid in the Yakima Valley of Washington. Frequent applications are required since demeton at this dosage is not effective for more than a week. Relatively high dosages of either schradan or demeton may need to be applied to potatoes if the period during which little reinfestation by aphids occurs is to be extended appreciably. Although high dosages may delay reinfestation of the plants by wingless aphids, this may not prevent the spread of the leaf-roll disease by the winged aphids since death of these may not occur until after the aphids have fed and transmitted the virus.

Summary.—Under conditions of continuous reinfestation of potatoes by winged aphids, multiple applications of parathion sprays were as effective as schradan or demeton in reducing populations of wingless aphids the first week after application. Parathion and Isolan had little residual effectiveness whereas schradan at 16 ounces per acre and demeton at 6 ounces per acre remained moderately effective for 2 weeks and schradan at 32 and demeton at 12 ounces for 3 weeks. Leaf-roll spread occurred with all treatments. Residues up to 0.38 p.p.m. demeton and 0.59 p.p.m. schradan were found in the potato tubers at harvest time. However, these residues did not adversely affect rats and monkeys fed on the potatoes.

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[3] Personal Communication from Wayland J. Hayes, Jr., Technical Development Communicable Disease Center, U. S. Public Health Service, Savannah, Georgia, November 24, 1954.

TENTH INTERNATIONAL CONGRESS OF ENTOMOLOGY

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Toxicity of DDT to the American Cockroach When Lipid Content and Temperature are Varied[1]

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When two groups of American cockroaches are similarly exposed to DDT and then placed, one at a high and the other at a low post-treatment temperature, the resultant per cent mortality at the lower temperature is found to be much greater than that at the higher temperature (Vinson & Kearns 1952). This phenomenon is referred to as the "negative temperature coefficient" of DDT (Richards & Cutkomp 1946, Fan *et al.* 1948). The negative temperature coefficient of DDT has been demonstrated with several different insects (Lindquist *et al.* 1945, Fan *et al.* 1948, Vinson & Kearns 1952, Roth *et al.* 1953, Häfliger 1954a and b) where temperatures have been varied only at treatment and post-treatment periods.

Fan *et al.* (1948) found in their studies with mosquito larvae of *Aedes aegypti* that the negative temperature coefficient of DDT occurred with immersion of the larvae in dilute suspensions of the insecticide. A positive temperature coefficient occurred if DDT emulsions were injected into the larvae or if they were immersed in more concentrated suspensions of DDT. Therefore, they proposed that the effect could be due, in part, to the DDT-concentrating properties of the cuticle at lower temperatures. Following the concentrating mechanism, the DDT would be eluted at the cuticle-cell interface to act at some vulnerable site. However, with the American cockroach, Vinson & Kearns (1952) found that the negative temperature coefficient still persisted after solutions of DDT in dioxane were injected, a technique which presumably minimizes the effect of the cuticle. They also presented data to show that the effect could not be accounted for as a result of differences in rates or magnitude of detoxification of DDT at the two temperatures.

The studies in this paper were undertaken in order to (a) test a possible explanation of the negative temperature coefficient as the effect might be related to injected doses of DDT in relation to quantity of lipids in the cockroach at different post-treatment temperatures and (b) to determine more about the relation of different quantities of insect lipids to the toxicity of DDT in general.

Very little is known about the DDT-lipid relationship in insects although a large volume of literature has developed pertaining to the storage of DDT in the fat of vertebrates (Brown 1951, pp. 504–518). Lipids on insect cuticle have been studied in relation to DDT solubility and toxicity (Pradhan *et al.* 1952). There have been some studies relating DDT toxicity to internal lipids, however. Munson and Gottlieb (1953) showed a high positive correlation between the resistance of American cockroaches to DDT and their lipid content. Munson *et al.* (1954) have reported on preconditioning temperatures as they affect American cockroach resistance to DDT and have related this resistance to lipid content. Their study has some bearing on relations of total lipid content to resistance to DDT but differs from the present work in that it deals with preconditioning while we are more concerned with post-treatment temperature effects. Berim & Edelman (1949) stated that generally the fat content of two beetles, *Agelasti alni* (Family Galerucidae) and *Pseudophonus pubescens* (Family Carabidae) was directly related to their resistance to DDT; however, they found that even though the fat content was higher before overwintering and during egg development in the females, the resistance of these insects to DDT was lower. Increased metabolism was shown to occur during these periods. This would indicate a more rapid turnover of the fat and presumably a release of more DDT in a shorter time. In contrast to these conclusions, Reiser *et al.* (1953) concluded that there was no direct relationship between the seasonal increase in the lipid content of the boll weevil, *Anthonomus grandis*, and its resistance to chlorinated hydrocarbon insecticides. Their conclusion was supported by the fact that the boll weevils containing large amounts of lipids were also more resistant to non-fat soluble insecticides. A different interpretation of these data has been suggested, however (Munson *et al.* 1954).

METHODS AND MATERIALS.—The cockroaches used during the course of the experiments were obtained from two sources. One group was supplied by the Public Health Department of the University of Minnesota and the other group by the Department of Entomology at Rutgers University. The cockroaches from these sources had no previous contact with insecticides. During the rearing and testing procedures the two groups were treated separately. Since the two strains did not differ appreciably in their susceptibility to DDT, the data from both groups were pooled for analysis.

The rearing method was essentially the same as that described by Heal (1948). Egg capsules were collected from the refuse under the adult cages and placed in a 6×8 inch battery jar. The young nymphs were removed at 10-day intervals, placed in clean battery jars provided with fibre-board platforms, and given dog food and water.

The total lipid content of a given group of cockroaches was modified by using different diets. This dietary modification was made more than 2 months before any insecticide exposure. Special diets were used in preference to other possible methods such as starvation. Groups of cockroaches were randomly divided and placed on one of three diets at the time a few cockroaches in the colony had reached the adult stage. The cockroaches were left on the diets for approximately 80 days before testing. The diets used were suggested by Haydak (1953) and consisted of dietary extremes of protein and carbohydrate

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Table 1.—Composition of diets used to vary total lipid content.

Diet	Per Cent of Casein	Dextrin	Dried Brewer's Yeast	Salt Mixtures	Per Cent Protein
Low Protein	0	91	5	4	2.5
High Protein	86	0	10	4	91.0
Dog Food[a]					24.0

[a] Ralston Purina product (Checkers). Composition: Crude protein, more than 24%; fat, more than 5%; fiber, less than 5%; and nitrogen free extract, more than 46%. Complete nutrient composition available from Ralston Purina Company.

(see Table 1). Dog food was used as the normal diet for comparison.

After completion of the conditioning period, the male and female cockroaches from each of the diets were separated, and each group was randomly divided into two smaller groups. One group was used for testing at 15° C. and one group for testing at 30° C. Solutions of DDT in acetone were injected into the abdomen of the cockroach (method similar to Heal & Menusan 1948). An ultramicroburet fitted with a No. 27 hypodermic needle was used. Volumes of acetone containing DDT ranged from 0.3 cubic millimeters to 2.0 cubic millimeters per cockroach. This was done to get a range of doses of DDT. No mortality occurred with these volumes of acetone when used alone with the exception of some cockroaches on the high protein diet. An occasional death occurred in cockroaches on the high protein diet when injected acetone exceeded 1.0 cubic millimeter. Because of this, the doses of acetone were kept below 1.0 cubic millimeter to this particular group of cockroaches. The group of cockroaches kept on the high protein diet were more sensitive to high temperatures (35° C.) and were the most susceptible to DDT at both post-treatment temperatures. However, the toxicity data from this group were homogeneous on the basis of the Chi² test, and the variation was not extreme (see Table 2 for confidence limits).

Each cockroach was weighed at the time of injection. Following injection, the cockroaches were placed in petri dishes with filter paper on the bottom, provided with proper diet and water and placed at the selected post-treatment temperature. Mortality was determined at 24-hr. intervals over a 5-day period.

In order to evaluate the effectiveness of the diets and to give a more complete picture of the relationship between the cockroach lipids and DDT, fat extractions were made on a number of individual cockroaches. Each cockroach was cut up and dried in a vacuum oven for 24 hours at 60° C. After obtaining the dry weight, extraction of lipids was made in a Soxhlet extractor using ethyl ether for 12 hours. Following drying, the weight of the dissolved lipids was determined.

Results.—The data presented include toxicity responses pooled from a series of five different toxicity experiments in which male and female cockroaches from each of the three diets were tested. The statistical method of Litchfield & Wilcoxon (1949) was used to make the analysis. The average dose and per cent mortality for each group of four or five cockroaches was calculated. The data are presented in figure 1.

Both sexes of cockroaches were more susceptible to DDT when they were exposed to a low post-treatment temperature (15° C.). This was consistent with all three diets (see Figures 1 and 2). Using the dog food diet as an example, the male cockroaches had an LD_{50} of 1.7 micrograms of DDT per gram of body weight at 15° C. in contrast to an LD_{50} of 10.5 micrograms per gram at 30° C. This represents a dose six times as great at the 30° C. temperature. A similar comparison with female cockroaches shows a difference of more than 10 times at the two temperatures. The LD_{50} values in this instance are 5.3 and 58.0 micrograms of DDT per gram of body weight at post-treatment temperatures of 15° and 30°C., respectively.

Additional observations revealed that cockroaches injected with DDT and showing no symptoms at 30° or 32° C. would elicit typical DDT tremors within minutes

Table 2.—The average dry weight and lipid content, and DDT toxicity values from injection of American cockroaches fed from 11–12 weeks on three different diets designed to produce three different lipid levels.[a]

Diet	Post-Treatment Temperature (° C.)	Number of Roaches Extracted	Number of Roaches for Toxicity	Average Dry Weight of Roaches (in Mgs.)	Lipid Values Mgs. per Roach	Lipid Values Per Cent (Dry Wt. Basis)	Acute Toxicity µg./g. Roach (LD_{50}) with Confidence Limits (19/20)
			Males				
Low Protein	15°	29	54	189.8± 4.0	18.0±1.6	9.5	1.0 (0.8– 1.1)
High Protein	15°	22	35	325.4± 9.0	13.8±1.0	4.2	0.4 (0.3– 0.5)
Dog Food	15°	21	42	233.8±10.2	34.0±3.7	14.5	1.7 (1.3– 2.2)
Low Protein	30°	36	27	162.9± 5.3	10.3±1.3	6.3	9.0 (6.0–13.5)
High Protein	30°	24	34	312.6±15.9	11.9±1.0	3.8	5.9 (3.9– 8.8)
Dog Food	30°	37	40	188.4± 4.7	14.8±1.8	6.6	10.5 (7.3–15.2)
			Females				
Low Protein	15°	43	63	225.1± 7.1	26.0±2.7	11.6	3.1 (2.2– 4.4)
High Protein	15°	25	46	474.2±21.2	14.5±0.9	3.1	1.2 (0.7– 1.8)
Dog Food	15°	28	54	311.6± 7.1	43.0±3.4	13.9	5.3 (4.2– 6.6)
Low Protein	30°	64	58	208.1± 6.0	18.2±1.5	8.7	41.0 (33.3–50.5)
High Protein	30°	42	44	475.6±13.9	13.5±0.7	2.8	21.0 (13.8–31.9)
Dog Food	30°	38	31	284.1± 7.7	31.9±2.7	11.2	58.0 (43.0–78.3)

[a] The toxicity data are shown in the regression lines in figures 1 and 2. Values following plus or minus signs are standard errors.

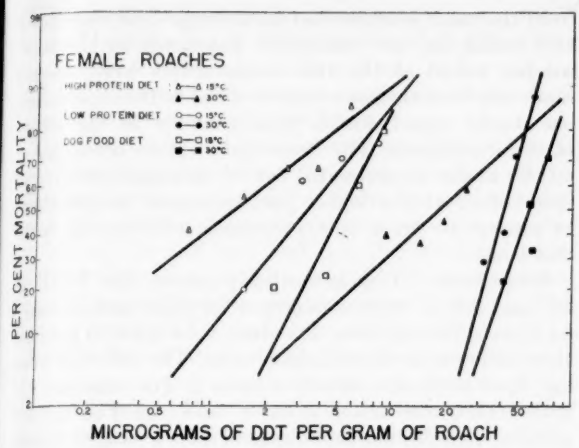

FIG. 1.—A comparison showing responses at two different post-treatment temperatures when DDT is injected into the abdomen of female American cockroaches fed for 11–12 weeks on three different diets designed to produce three different average lipid levels.

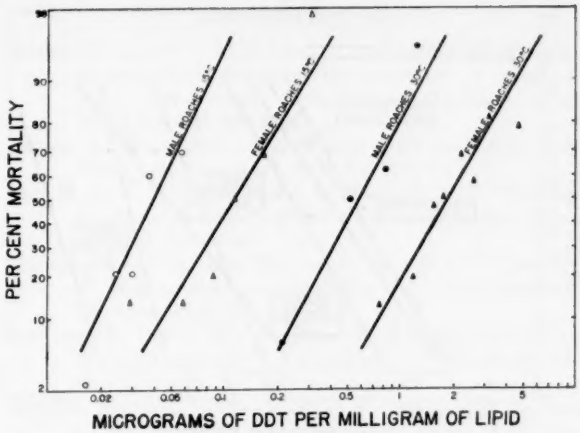

FIG. 3.—A comparison showing responses at two different post-treatment temperatures when DDT is injected into male and female American cockroaches. The dosage is calculated as micrograms of DDT per milligram of lipid. Each regression line represents data from the same individuals used to establish the regression lines in figures 1 and 2 where dosage was calculated as micrograms of DDT per gram of cockroach.

when the temperature was lowered by as little as 5°. Furthermore, the symptoms could be made to disappear at 30° C. and return (at 20° and 25° C.) more than a dozen times in some individuals. In most cases these observations were carried out over a period of 2 to 3 weeks.

The greater susceptibility of male cockroaches in comparison to female cockroaches as indicated in the example above (1.7 vs. 5.3 and 10.5 vs. 58.0 micrograms of DDT per cockroach) was evident on all diets and at both temperatures (Table 2).

The high protein diet produced cockroaches with a greater susceptibility to DDT than those reared on the low protein or the dog food diet. The cockroaches reared on a dog food diet were the least susceptible. These results were consistent at both post-treatment temperatures.

The lipid content resulting from the three diets showed considerable variation (Table 2). Furthermore the variable susceptibility of cockroaches fed on different diets could be due to the diets or the lipid content. The lipid contents were studied relative to responses to DDT by graphically presenting dosages of DDT per milligram of lipid per cockroach. The data from all three diets were pooled for this analysis (Figure 3). Using the same individuals, the data were analyzed from the standpoint of DDT per gram of body weight (Figure 4). This provided a comparison between the two methods of expressing dosage. In those diet, sex and temperature groups where an analysis of the data was possible, regression lines were plotted both on the basis of DDT per unit of body weight and DDT per milligram of lipid per cockroach (Figure 5). In general, the range of dosages was greater when plotted

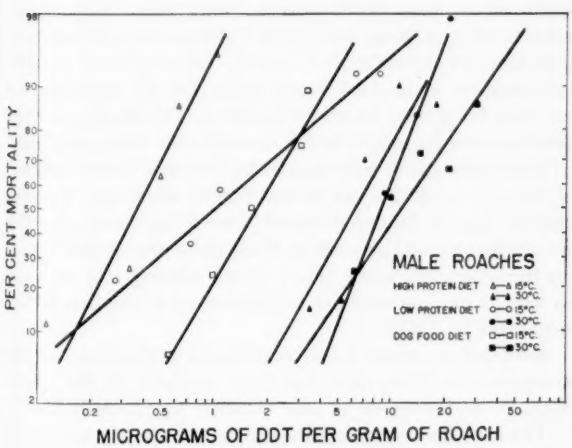

FIG. 2.—A comparison showing responses at two different post-treatment temperatures when DDT is injected into the abdomen of male American cockroaches fed for 11–12 weeks on three different diets designed to produce three different average lipid levels.

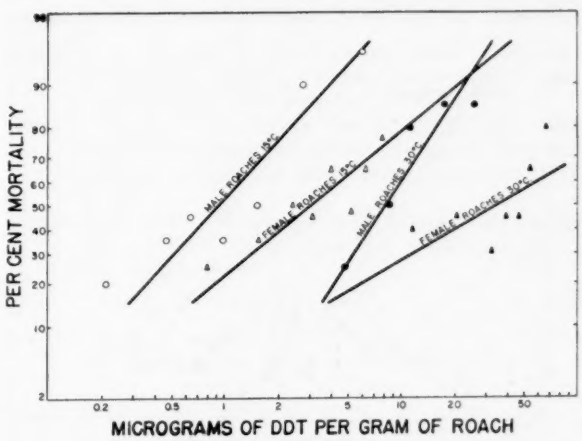

FIG. 4.—A comparison showing responses at two different post-treatment temperatures when DDT is injected into male and female American cockroaches. The dosage is calculated as micrograms of DDT per gram of cockroach. Data is based upon the same cockroaches as in figure 3.

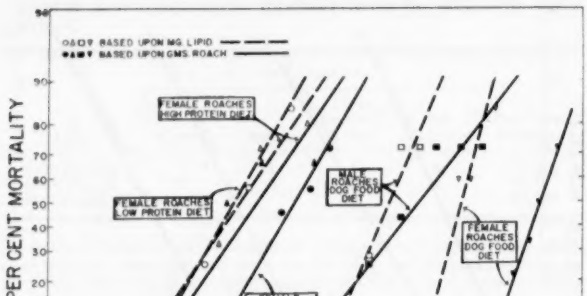
PER CENT MORTALITY
FEMALE ROACHES HIGH PROTEIN DIET
FEMALE ROACHES LOW PROTEIN DIET
MALE ROACHES DOG FOOD DIET
FEMALE ROACHES LOW PROTEIN DIET
FEMALE ROACHES DOG FOOD DIET
15°C.
30°C.
MICROGRAMS OF DDT
PER MILLIGRAM OF LIPID (UPPER SCALE)
PER GRAM OF ROACH (LOWER SCALE)

FIG. 5.—A comparison of DDT effects using the two methods of presenting response data. The results include only those diet and temperature groups where sufficient data were available for both methods.

against body weight than when plotted against lipid values. This is apparent by differences in slope of the regression lines.

The results show that when lipid content is high, more DDT is required to produce a given per cent mortality. A similar trend is apparent when the data are expressed on a body weight basis. Thus, a close relationship between the body weight of the cockroach and its lipid content is indicated. By plotting the body weights of the cockroaches versus lipid contents, a positive correlation appears to exist (consult Figure 6). However, the variation was so great that a precise analysis could not be made.

Table 2 shows the lipid content of cockroaches tested at 15° C. to be consistently higher than that from cockroaches tested at 30° C. For example, the total lipid content for male cockroaches at 15° C. is 34.0 milligrams per cockroach, while at 30° C. cockroaches which were taken from the same colonies had an average lipid content of 14.8 milligrams per cockroach. Inasmuch as the cockroaches tested at the two temperatures were selected randomly from the same colonies prior to the experiment, one would expect similar lipid contents at the outset of the experiment. The lower lipid values occurring at 30° C. might be accounted for by increased activity or metabolism at the higher post-treatment temperature; at present we have no experimental evidence to verify this point.

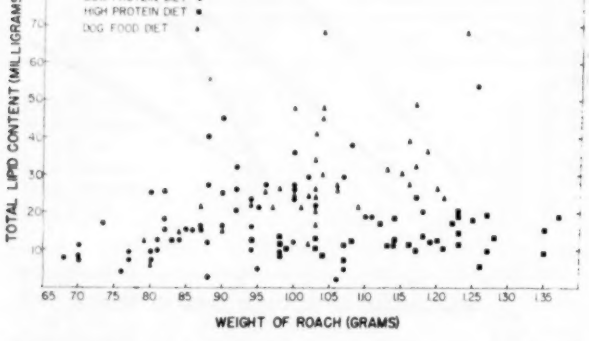

FIG. 6.—The total lipid content of female American cockroaches plotted against their body weight. The heterogeneity of the data was greatest on the dog food and low protein diets.

DISCUSSION.—Dosage-mortality curves for DDT at 15° and 30° C. were established for cockroaches reared on three different diets. The diets were used to produce three different levels of lipid content. The different average lipid levels are shown in table 2. For example, the male cockroaches tested at 15° C. and reared on the low protein, high protein, and dog food diets averaged respectively, 18.0, 13.8, and 34.0 milligrams of lipid per cockroach. However, there was an overlapping of the individual lipid contents of the cockroaches on all three diets, especially on the low protein and high protein diets. Using the example of male cockroaches tested at 15° C. again, the range of the lipid contents in milligrams of lipid per cockroach were as follows: Low protein, 5.8 to 40.0; high protein, 3.7 to 23.6; dog food, 16.7 to 82.0.

The lipid extractions revealed that cockroaches of the same sex and fed the same diet had a consistently higher lipid level at 15° C. than at 30° C. Since lipid variations occurred the customary method of plotting data, that is, mortality versus microgram of DDT per gram of cockroach, was not completely satisfactory for studying the effect of the total lipid content upon the toxicity of DDT. A better evaluation of the importance of lipids appeared possible by presenting the data as per cent mortality versus micrograms of DDT per milligram of lipid (see Figure 3).

A greater homogeneity of data is evident when plotted on the basis of DDT per milligram of lipid; that is, the range of dosages between dead and alive cockroaches is narrower. As a result, a large part of the data based upon body weight fell into the 100% and 0% mortality groups when based upon lipid content. Since there did not appear to be any large variations between the cockroaches fed the three different diets, all the data for each sex and temperature were pooled and analyzed. In addition, regression lines were plotted (Figure 5) for those diet and temperature groups in which enough data were available.

The influence of one particular dietary factor cannot be readily evaluated from the results obtained. For example, the cockroaches reared on a high protein diet weighed more on the average than the cockroaches reared on the other two diets. The factors causing this increase in weight may or may not be influential in relation to the responses to DDT.

Keeping in mind these comments pertaining to the analysis, the diets and the lipid content of the cockroaches, a discussion of the toxicity results will follow.

The toxicity data presented in figures 1 and 2 and table 2 show that DDT is consistently more toxic at post-treatment temperatures of 15° C. than at 30° C. The magnitude of the difference in toxicity, however, varies from group to group. For example, using the LD_{50} value

DDT is shown to be 16 times as toxic at 15° C. as at 30° C. for male cockroaches on a high protein diet. This difference is only 6.5 times for male cockroaches fed the dog food diet. The other series of cockroaches are intermediate in response between these two figures.

The lipid content of the cockroaches on two different diets does not appear to be a factor which influenced the differences in toxic levels given above. In the above example the toxicity differences at the two temperatures are less on the dog food and this diet produced cockroaches with greater quantities of lipids. This may be due to nutritional differences but without supporting data does not lend itself to a ready interpretation.

Despite the variations which occurred in the lipid values, the results show that cockroaches having low levels of lipids gave no indication of reversing or even minimizing the negative temperature coefficient effect of DDT. Conversely, there was no trend indicating that cockroaches with higher lipid contents had a wider range of sensitivity to DDT between 15° C. and 30° C. This does not preclude the possibility that cockroaches with a negligible lipid content (below 10 mgs. lipid dry weight) might not respond to the temperature difference positively instead of negatively. However, the data do not indicate any such trend. The lack of such a trend is quite apparent in figure 3 where the dosage-response curves are nearly parallel. However, the results do not rule out the possibility that qualitatively, the type of lipid might be important in the negative temperature coefficient relationships.

SUMMARY AND CONCLUSIONS.—Dosage-mortality responses to injected DDT were determined for male and female American cockroaches subjected to post-treatment temperatures of 15° and 30° C. The cockroaches were selected from three different dietary groups, a high protein, a low protein and a "standard" dog food. The results are considered from the standpoint of dietary influence and total lipid content of individual cockroaches.

A higher per cent mortality resulted from DDT at the lower post-treatment temperature and in all cases males were more susceptible than females. These results were similar on all three diets. Cockroaches fed on the high protein diet were more susceptible to DDT than cockroaches from the other diets. These cockroaches also had a lower lipid content. There was no indication that the quantity of lipids present was a factor which might be responsible for the negative temperature effect of DDT which occurs when cockroaches are subjected to contrasting post-treatment temperatures. Furthermore, the differences in quantity of lipids between sexes is not sufficient to explain sex differences in response to the action of DDT.

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Insects Lower the Quality of Cotton Lint and Seed[1]

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Insect attack on cotton has a serious detrimental effect on the quality of the lint and seed. It appears that heretofore this loss chargeable to insects has been underestimated and certainly has not been adequately measured. In general, gains from insect control have been measured in pounds of seed cotton or lint produced, with little attention to staple, grade, or seed quality. True, a number of entomologists have included in reports of results of insect control tests some mention of lint or seed damage.

Most of the published information on the effect of insect attack on cotton quality is not in detail but merely sets forth the sale price of the lint and seed of the cotton from plots or areas protected from insects by insecticides as compared with that from check areas.

Insects affect cotton from the time the seed is planted until the livestock eat the cottonseed cake. The insects that attack the crop in its early stages of development probably have no direct effect on the quality of cotton products but definitely affect yield. These insects include cutworms, darkling beetles, thrips, aphids, and cotton fleahoppers.

Insects that attack the plants after the bolls are set may materially affect the quality of lint and seed. This effect may come about in several ways. Some pests such as spider mites, aphids, and cotton leafworms may so defoliate the plants as to affect adversely normal maturity of the bolls which results in weak, short and wasteful lint and immature, poorly filled seed. Other insects that attack the bolls such as boll weevils, bollworms, pink bollworms, and hemipterous insects have long been recognized as injurious to the quality of cotton products.

Effect of Pink Bollworm on Cotton Quality.—In 1954, when the pink bollworm was not extremely abundant in the Port Lavaca, Texas, area, G. L. Smith of the Cotton Insects Section, U.S.D.A., conducted an insecticide test in which the cotton from plots treated seven times with DDT produced an average of 1403 pounds of snapped cotton per acre as compared with 841 pounds on the check, a gain of 562 pounds per acre. The sale price of the lint from the treated plots was 34.5 cents per pound as compared with 26.5 cents for that from the untreated ones. Furthermore, the lint yield from the treated plots was far higher than is indicated by the yields of snapped cotton, since that from the untreated plots contained more trash.

In the Lower Rio Grande Valley there was a much heavier infestation of pink bollworms in 1952 than during previous years. Some of the growers in this area reported little or no loss due to reduced grade while others averred their losses ranging from 5 to 30 dollars per bale for part of their crop. Cotton buyers reported a marked reduction in grade in 1952 over preceding years but were unable to make an estimate as to how much of the loss was due to pink bollworm damage and how much to other factors. It has been found in experimental work that a "spotty" grade is characteristic of cotton heavily infested by pink bollworms. It therefore appeared that records on the percentage of cotton samples that were spotted in 1952 compared to 1951 would indicate a damage factor attributable to the pink bollworm. The U.S.D.A. Production Marketing Administration cotton classers furnished records on spotted cotton at gins from which 75 per cent or more of the samples were received for classing. The percentage of spotted samples from the various gins ranged from 4.9 to 34.7, and averaged 14.4 per cent in 1952. In 1951 these figures averaged 0.4 per cent. It is believed the high percentage of spotted samples for 1952 can be largely attributed to pink bollworm damage.

The relation between percentage of pink bollworm infestation and percentage of stained lint is indicated by studies conducted in 1954 by Joel Hembree of Cotton Economic Research, University of Texas; the Pink Bollworm Control Project, U.S.D.A.; the Cotton Division, Agricultural Marketing Service; and the Texas Technological College (Anonymous, 1954). Cotton from five fields with different degrees of pink bollworm infestation, from 7 to 90 per cent, was gathered by snapping. The cotton was first divided into three classes: pickable, partially pickable, and non-pickable. The results are shown in table 1.

Some of the fiber properties of samples from three of these are shown in table 2. Note the marked effect of the heavier infestations on the fineness, maturity and yarn strength. The effect on value is also striking. Note the lowered classification and decreased value of the lint, $11.00 to $36.00, depending on degree of infestation as shown in table 3.

Lint from 100 bolls apparently undamaged and 100 bolls damaged by the pink bollworm from four of the farms mentioned in the previous discussion, was studied by Mr. Hembree. The fiber properties of this lint are presented in table 4. Mr. Hembree points out that the lint from the damaged bolls was 1/16 inch shorter, uniformity about 4 per cent less, tensile strength some 10,000 pounds less, fineness 1.2 units lower, and maturity approximately 14 per cent lower. The damaged cotton showed a lower reflectance and it was more yellow than the undamaged cotton.

In a large scale pink bollworm control experiment carried out at Robstown, Texas, in 1952 by C. A. Richmond of the Cotton Insects Section, U.S.D.A., the lint from the first picking in the area treated with insecticides brought 2 cents per pound more than that from the untreated area, and the lint from the second picking brought a difference of 8 cents per pound (Table 5). The percentage of trash in the snapped cotton was much greater in the untreated than in the treated cotton, being 31 and 57 per cent, respectively.

In some of the research carried out by A. J. Chapman and S. L. Calhoun of the Cotton Insects Section, U.S.D.A., in the pink bollworm infested area near Presidio, Texas, samples of the cotton were submitted to R. W. Webb of the then Bureau of Agricultural Economics and classed by H. C. Slade of that organization.

[1] Accepted for publication August 25, 1955.

[2] Retired as Coordinator, Pink Bollworm Research, Brownsville, Texas, on June 30, 1955.

Table 1.—Relation between pink bollworm infestation and percentage of lint stained.

Field No.	Infestation Level Per Cent	Kind of Cotton: Per Cent Pickable White	Per Cent Pickable Stained	Per Cent Pickable Contained[a]	Per Cent Partially Pickable White	Per Cent Partially Pickable Stained	Per Cent Partially Pickable Contained	Per Cent Non-Pickable White	Per Cent Non-Pickable Stained	Per Cent Non-Pickable Contained
1	7	76.7	6.8	1.7	6.8	2.3	1.7	0.6	1.1	2.3
2	39	55.9	10.7	1.9	15.4	5.4	4.2	5.4	0.6	0.6
3	65	54.0	14.8	1.6	6.6	6.0	6.6	2.2	2.7	5.5
4	67	42.4	19.4	1.2	7.9	16.5	3.6	0.6	3.6	4.8
5	90	15.2	33.9	1.3	9.3	24.5	7.9	1.3	4.0	2.6

[a] "Contained" refers to the cotton that did not come out of the boll with the force ordinarily required to pick cotton.

Table 2.—Pink bollworm infestation and fiber properties of cotton tested by the Economic Research Laboratory, University of Texas.

Properties	Per Cent of Cotton Infested: 7	65	90
Upper half mean[a]	0.92	0.87	0.88
Uniformity[b]	79	71	74
Tensile strength[c]	85	81	82
Fineness (micronaire)[d]	4	2.8	2.9
Maturity[e]	81	71	70
Yarn strength 22s[f]	86	78	79

[a] Average length of the longest half of the fibers by weight, corresponds with staple length as determined by the classer, and is expressed in decimal fractions of an inch.

[b] A measure of fiber length distribution, secured by dividing the mean by the upper half mean and expressing the result in per cent. Above 80 is considered uniform.

[c] Force in 1000 pounds required to separate the equivalent of a surface area of 1 sq. in. Ninety or above is considered excellent, 83 to 89 very good, 78 to 82 average, 72 to 77 fair, and below 72 is considered weak.

[d] Weight of 100 fibers as extracted from each length of an array in proportion to their relative weights in the array; 1.8 to 2.9 micrograms per inch is considered extra fine, 3.0 to 3.9 fine, 4.0 to 4.9 average, 5.0 to 5.9 coarse, and above 6.0 is considered very coarse.

[e] Percentage of thick-walled fibers. Eighty-four or above is considered very mature, 77 to 84 mature, 68 to 76 average, 60 to 67 immature, and 60 or below very immature.

[f] Strength in pounds of relatively coarse yarn designated "22s."

Mr. Slade stated that in some instances a part of the samples were so heavily damaged that that portion was below any grade of the Universal Standards. His classification also showed the fibers to be light and very weak, especially in the case of the last picking.

In experiments conducted at Presidio, Texas, by O. T. Robertson of the Cotton Insects Section, U.S.D.A., in 1946, a gain of 900 pounds of seed cotton was obtained by the use of insecticides against the pink bollworm. The lint from the treated plot was classified as "good middling extra white" with a staple length of $1\frac{1}{8}$ inches. The cotton in the untreated section was practically destroyed. Only 351 pounds of seed cotton were produced. The lint was classified as "good ordinary irregular, weak and wasty" with a staple length of 13/16 inches. No commercial price quotation could be obtained for lint of this grade.

The point should be made that many of the severely damaged bolls are not picked. This applies to bolls damaged by bollworms and boll weevils as well as to the pink bollworm. In some experiments it has been found that yield was not lowered by a very light infestation of the pink bollworm. It seems certain, however, that such an infestation would lower the lint quality; furthermore, such light infestation occurring during wet seasons may lower yield as well as quality through increased boll rot. The injury to bolls produced by the pink bollworm, boll weevil, and bollworm gives opportunity for fungi and bacteria to enter and cause the lint to be stained and weakened or the bolls to rot.

EFFECT OF PINK BOLLWORM ON SEED.—In addition to the effect of the pink bollworm on cotton lint, there is an important effect on the seed. This is shown on table 6 where, according to a study conducted in the Lower Rio Grande Valley by A. J. Chapman and K. P. Ewing, seed turnout per bale during the heavy pink bollworm season of 1952 is compared to that of 1951 when the infestation was relatively light. The average seed turnout per bale in the 1951 crop was 850 pounds as compared with 778 pounds in the 1952 crop, a loss of 71.7 pounds. On the basis of $75.00 per ton for cotton seed, this would give a

Table 3.—Reduction in the value of cotton per bale from lowering of grade and reduction of staple length as a result of pink bollworm damage.

Per Cent Infestation	Control: Grade	Staple	Value	Blend: Grade	Staple	Value	Difference in Value
7	M-W[a]	15/16″	$166.10	SLM-W	29/32″	$155.05	$11.05
39	SLM-W[b]	29/32″	155.05	SLM-W	29/32″	155.05	0
65	M-W	15/16″	166.10	SLM-W	29/32″	155.05	11.05
67	M-W	31/32″	167.80	SLM-S[c]	29/32″	131.15	36.65
80	M-W	31/32″	167.80	SLM-W	29/32″	155.05	12.75
90	M-W	31/32″	167.80	SLM-S	29/32″	131.15	36.65

[a] Middling white.
[b] Strict low middling white.
[c] Strict low middling stained.

Table 4.—Comparison of fiber properties of undamaged cotton and cotton damaged by the pink bollworm.

Fiber Property	100 Undamaged Bolls from Farm				100 Damaged Bolls from Farm			
	A	B	C	D	A	B	C	D
Upper half mean[a]	0.86	0.95	0.99	0.94	0.83	0.86	0.83	0.88
Uniformity[a]	74	76	75	79	72	73	70	73
Tensile strength[a]	78	87	81	87	65	80	78	64
Fineness[a]	5.5	3.7	3.4	4.1	3.3	2.7	2.4	3.5
Maturity[a]	85	79	72	80	66	65	57	71
Reflectance[b]	75	71	70	72	62	65	58	60
Degree of yellowness[c]	10.0	10.5	11.3	11.1	12.4	11.3	11.4	11.9
Yarn strength 22s[a]	69	84	92	89	58	75	69	65

[a] For definition, see footnote table 2.
[b] Relative lightness or darkness of color in terms of per cent.
[c] Degree of yellowness on Hunter's scale of +b.1.

loss of $2.69 per bale. Similar information obtained from counties north of the Valley indicated a loss of $1.35 per bale. These figures applied to the 1952 crop gives a seed loss at the gin of $1,388,061.

Not only is there a loss in seed yield of 71.7 pounds of seed per bale in the 1952 crop over the 1951 crop in the Lower Rio Grande Valley, but there is also a corresponding loss in cotton seed products. Information furnished by oil mills in the heavily infested area indicates there was a loss per ton of cotton seed of 27 pounds of oil, 9 pounds of cake, 5 pounds of first cut linters and 38 pounds of waste or a total loss per ton of seed from the 1952 crop of $5.93. Applying this figure to the crop in the heavily infested area of south Texas, we have an estimated loss of $1,298,472.

Effect of Other Insects on Lint Quality.—Marked detrimental effects on cotton lint are produced by a number of insects in addition to those just mentioned. In some areas cotton aphids become very abundant and the honeydew excreted by them seriously contaminates the lint. This sticky material, consisting of reducing sugars—sucrose, dextrin, and other carbohydrates, interferes with spinning and induces the growth of molds on open bolls which stain and weaken the fiber. Aphid infestations developing late in the season are usually disregarded by the growers because they do not have an appreciable effect on total yield. They may, however, cause the growers to pay a premium for picking as pickers object to working in cotton drenched with honeydew. The growers also lose because the mills object to honeydew and a community may suffer from its reputation for producing cotton contaminated by honeydew. A qualitative test for honeydew on raw cotton has been developed by Stansbury & Hoffpanir (1950).

The effect of defoliation by the cotton leafworm appears not to have been carefully evaluated. Early defoliation by this insect would appear to be somewhat similar to the effect of premature chemical defoliation, though perhaps not quite as severe as some leaves and leaf veins are usually left on the plants. The ill effect of leafworm defoliation does not stop with the leaf removal. Probably the major damage results from the green stain on the lint caused by excrement of the larvae. This damage is well recognized. Showers and heavy dews occurring while the leafworms are at work intensifies the trouble from stains.

The cotton leaf perforator, *Bucculatrix thurberiella* Busck, is usually regarded as a minor cotton pest; however under conditions of heavy infestation, it may adversely affect both yield and fiber quality. E. E. Berkley, William Blackledge, and R. E. Beckett of Anderson Clayton & Company have kindly permitted me to use some interesting information gathered in northwestern Mexico and at the Company's laboratories in Houston regarding the damaging effect on cotton of this insect.

On one farm the perforator was controlled with DDT-toxaphene-sulfur 5-15-40 at 25 pounds per acre and a yield of 1.177 bales per acre obtained whereas on the other

Table 5.—Effect of pink bollworm on cotton yield and value, Robstown, Texas, 1952.

	Cotton Yield and Value per Acre			
	First Picking		Second Picking	
	Check	Treated[a]	Check	Treated[a]
Pounds snapped bolls	1579	1646	934	956
Per cent lint	25	25	16	25
Per cent seed	44	44	27	44
Pounds of lint	395	412	149	239
Price per pound (lint)	$0.38	$0.40	$0.30	$0.38
Total value of lint	$150.00	$165.00	$45.00	$91.00
Value of seed	$26.00	$27.00	$9.00	$16.00
Total gain over check	—	$16.00	—	$53.00

[a] Treated area partially protected by insecticides.

Table 6.—Loss in seed yield at gins in the Lower Rio Grande Valley of Texas for 1952 crop as compared with 1951 crop.

Gin No.	Year	No. Bales Ginned	Pounds of Seed per Bale		Tons Seed Lost 1952
			Yield	1952 Loss	
1	1951	2717	802		
	1952	1045	728	74	38.7
2	1951	4837	887		
	1952	2914	834	53	77.2
3	1951	5532	870		
	1952	2837	794	76	107.8
4	1951	3279	840		
	1952	1537	756	85	64.5

Table 7.—Spinning tests on cotton from a field infested by the cotton leaf perforator and one where the perforator was controlled, San Luis, Mexico.

Insect Infestation	Class	Fiber Length (Fibrograph)	Fiber Strength (Pressley)	Fiber Fineness (Micronaire)	Picker & Card Waste[a]	Card Web Appearance			Yarn						
						Neps[b] per Grain	Neps per 100 Sq. In.	Trash per 100 Sq. In.	Skein Strength			Skein Appearance			Grade Index
		Upper Half Mean							8s	14s	22s	8s	14s	22s	
		Inches	*1000 Lbs./ Sq. In.*	*Reading*	*Per Cent*				*lbs.*	*lbs.*	*lbs.*				
Untreated	Strict low middling to low middling-light spot—7/8"	0.96	86	3.2	10.8	7.9	22	16	333.3	185.8	112.7	C+	C+	C	98
Treated	Low middling-extra white—15/16"	1.08	85	4.8	11.1	4.3	12	6	342.0	180.9	114.8	B	B	B	110

[a] Includes Super-Jet Cleaner Waste.
[b] Minute tangled masses of fibers

farm where the heavy perforator infestation was not controlled, the yield was only 0.275 bale per acre. The treated area, however, had received 66 pounds of nitrogen per acre in the form of urea. Both areas were planted May 15, 1954, to Deltapine 15 cotton and harvested November 26. You will note from table 7 that the sample from the perforator-controlled area showed higher grade, longer staple, higher micronaire, fewer neps, less trash, somewhat greater yarn strength and higher grade index. Although this information is based on one test, it indicates that a heavy infestation of the perforator seriously affects the quality of the lint.

Plant feeding bugs, such as stink bugs and cotton stainers, often pierce full grown bolls with their beaks and introduce disease organisms by these punctures. These insects are especially abundant in west Texas, New Mexico, and Arizona where the damage inflicted is often considerable. Not only do these bugs increase trouble from boll rot, but they also cause reduction in the quality of lint and seed, as has been shown in work carried out by T. P. Cassidy (U.S.D.A.) in Arizona, as reported by Annand (1942). The improvement in quality of long-staple cotton from the control of plant bugs gave returns ranging from $2.85 to $30.00 per bale. The effect of these insects on cotton quality, however, has not been fully evaluated.

Considerable interest has been manifested recently in fluorescence appearing on cotton lint viewed under ultra violet light and the relation of that phenomenon to cotton quality. The low intensity fluorescence produced by honeydew has been used for several years as an aid in detecting cotton so contaminated. Fluorescence has been definitely associated with fungus contamination, especially by *Aspergillus flavus*, according to Bollenbacher & Marsh (1954). This type of fluorescence is evident in lint from pink bollworm infested bolls and some of the larvae themselves are strikingly fluorescent under ultra violet light. This indicates that the larvae are carrying the fungus which they undoubtedly transport into bolls penetrated by them. Weakness of lint is associated with fluorescence and such lint may not dye uniformly. The introduction of fungi is also facilitated by the exit holes of the pink bollworm larvae and exit or entrance holes made by boll weevils and bollworms.

EFFECTS OF INSECTS ON COTTON SEED PRODUCTS IN STORAGE.—Cottonseed is an important source of food for man and livestock and since practically all stored product insects will attack the broken cotton seeds, cottonseed cake and meal, they produce considerable losses.

According to Back (1939), all storage pests are likely to be found upon extended search around stored cottonseed meal, but only the cigarette beetle, *Lasioderma serricorne* (F.), and possibly, under protracted periods of storage, mealworms, *Tribolium* spp., are of importance. The cadelle, confused flour beetle, and some of the flour moths are commonly found in stored cottonseed cake and meal. The recently introduced khapra beetle, *Trogoderma granarium* Everts, has been found infesting cottonseed meal and stored cottonseed where it appears to attack only broken seeds.

The direct damage to cottonseed products from these pests is not great. However, shipments of heavily infested material have been refused and cost of fumigation to hold down infestations are considerable. Furthermore, heavy infestations of the cigarette beetle in sacked meal destroy the sacks and increase the cost of handling the product.

Other complicating factors which must be considered in dealing with cotton insect problems is the possible contamination of cottonseed products by insecticides used to control the pests; also possible staining or other adverse effects of insecticides or defoliants, used in connection with insect control, on the quality of the lint. Another problem which has presented itself, but which thus far is not serious, is the adverse effect on the growth or quality of cotton of accumulations of insecticide residues in the soil.

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Biological Control of Fruit Flies[1]

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The fruit flies of the family Tephritidae are destructive pests of a wide range of fruit and vegetable crops throughout the world and are of greatest importance in tropical and subtropical areas. The biological control of these pests has received special attention in Hawaii because of the occurrence there of three of the major crop pests of the group, the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), the melon fly, *Dacus cucurbitae* Coq., and the oriental fruit fly, *Dacus dorsalis* Hendel. Research on these problems began in 1912, when the Hawaii Board of Agriculture and Forestry undertook the search for natural enemies of the Mediterranean fruit fly. The Italian entomologist, F. Silvestri, was employed for this purpose and during 1912–13 made collections in West and South Africa and in Australia (Silvestri 1914). His efforts resulted in the establishment in Hawaii of two larval parasites, *Opius humilis* Silv. from South Africa and *O. tryoni* (Cam.) from Australia, as well as a pupal parasite, *Dirhinus giffardii* Silv. from West Africa. In 1914, D. T. Fullaway and J. C. Bridwell went to West Africa to obtain other species previously reported by Silvestri, and succeeded in importing and establishing *O. fullawayi* Silv. and a second pupal parasite, *Tetrastichus giffardianus* Silv.

In 1935–36, the Federal Bureau of Entomology and Plant Quarantine undertook further investigations, sending expeditions to West and East Africa, Brazil, Malaya and India. Unfortunately, this project was prematurely terminated because of lack of funds and none of the parasites imported during the short period of search became established. The final results, in terms of field control, are therefore based on the three species of *Opius* previously mentioned.

The interrelations of these parasites and the results of competition between them have been the subject of detailed study (Pemberton & Willard 1918). *Opius humilis* attained a high parasitization very quickly after initial release, but was soon superseded by *O. tryoni*, and the latter species retained its dominance continuously thereafter. Field parasitization over a 20-year period, 1914–33, averaged 42.3 per cent in all fruits (Willard & Mason 1937). In coffee berries in the Kona district of the Island of Hawaii, the parasitization ranged from 45 to 95 per cent during this period. This high incidence of attack, due to the accessibility of the larvae to the parasites throughout their period of development, resulted in a marked reduction in the field infestations in coffee, amounting to more than 90 per cent in some years. Equally high parasitization also occurred in Indian almond, or kamani, *Terminalia catappa* L. The effect on the pest in other fruits, especially those having a deep pulp, was much less, parasitization of larvae in mango, for example, ranging only from 6 to 26 per cent and in guava from 5 to 23 per cent.

Of the two established pupal parasites, *Dirhinus giffardii* is found only occasionally, while *Tetrastichus giffardianus* is of negligible importance except against the pest in certain fruits, such as orange and yellow-oleander.

Melon Fly.—Foreign exploration for natural enemies of the melon fly was undertaken in Malaya and India by the Hawaii Board of Agriculture and Forestry in 1915–16 and resulted in the finding of *Opius fletcheri* Silv. in India and its importation and establishment in Hawaii (Fullaway 1919). Field parasitization soon reached the 50 per cent level but declined thereafter, and the parasite, while still common, now has little or no effect on the pest infestations. It reaches its peak of about 40 per cent parasitization during the winter but declines to 10 per cent or less during the summer months. *Opius watersi* Full. from North India and Ceylon and *O. angaleti* Full. from Borneo, both of which are from the melon fly and were imported into Hawaii during 1950–51, failed to become established.

Oriental Fruit Fly.—The appearance of the oriental fruit fly in Hawaii about 1946 and its very rapid increase and spread led to the initiation of foreign investigations by the Territorial Board of Agriculture and Forestry in 1947. This was followed by the large-scale program of the Federal Bureau of Entomology and Plant Quarantine, beginning in 1948, and by the University of California in 1949. The entire foreign exploration phase of the fruit fly project extended from July, 1947 to September, 1951, during which period 14 collectors were in the field for various lengths of time. Most of the tropical and subtropical areas of the world were covered, with special attention to Malaya, India, East, West and South Africa, Australia, and the Pacific Islands (Clancy *et al.* 1952).

The puparia of all available fruit flies were collected and forwarded to Hawaii for rearing and testing of the parasites under quarantine. These shipments comprised a total of more than 4,246,000 puparia, of which more than 1½ million were of *Dacus dorsalis*, with a small number of immature and adult parasites and predators. They comprised more than 60 species of Tephritidae reared from 110 different kinds of fruits, and from them 80 or more species of parasites were obtained. Sixteen or more species and varieties of larval parasites of the genus *Opius*, 6 pupal parasites, and 1 predator were released in the field in Hawaii. A total of 1,419,777 of these 23 species have been released by the Territorial Board of Agriculture and Forestry, which was responsible for the rearing and colonization program, from 1948 to 1955. The 11 species and varieties known to be established are as follows:

Species	Native Host	Country of Origin
Opius compensans (Silv.)	*Dacus dorsalis*	India
formosanus (Full.)	*Dacus dorsalis*	Formosa
incisi Silv.	*Dacus dorsalis*	Malaya, India, Borneo, etc.
krausii Full.	*Dacus dorsalis*	Australia
longicaudatus (Ashm.) var. *malaiensis* Full.	*Dacus dorsalis*	Malaya, Borneo, India
var. *novocaledonicus* Full.	*Dacus* spp.	New Caledonia
var. *taiensis* Full.	*Dacus* spp.	Thailand
oophilus Silv.	*Dacus dorsalis*	Malaya, India, Borneo, etc.
vandenboschi Full.	*Dacus dorsalis*	Malaya
Syntomosphyrum indicum Silv.	*Dacus* spp.	Malaya, etc.
Tetrastichus dacicida Silv.	*Dacus* spp., *Pardalaspis* sp., and *Pterandrus* sp.	Kenya

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Opius longicaudatus malaiensis increased rapidly in the field after its initial release in 1948, but was superseded the following year by *O. vandenboschi*. *O. oophilus* then became the dominant parasite in 1950 (van den Bosch *et al.* 1951), attaining a parasitization on the island of Oahu of 70 per cent or more, and has maintained this high level since that time. *O. longicaudatus malaiensis* and *O. vandenboschi* subsided to a very low level and, with the remaining established species of *Opius*, now contribute little to control. *O. oophilus* oviposits in the host egg, *O. vandenboschi* in the first-instar larvae, and *O. longicaudatus* in the second- and third-instar larvae. The different host relationships may explain the dominant role assumed by *O. oophilus*. In addition to the host mortality resulting from direct parasitization, the females of this species serve as carriers of bacteria and fungi that destroy a considerable portion of the host eggs.

The net result of the biological control program in Hawaii has been a very substantial reduction in the oriental fruit fly populations in most parts of the Islands. Average parasitization based on emergence records is in the neighborhood of 70 to 75 per cent, to which may be added the substantial mortality of eggs resulting from disease transmitted by the parasites. Field populations in most localities on Oahu are now only a small fraction of those present prior to 1949, while on other islands, especially some areas on Hawaii and Maui, the reduction has not been so great. Control is far from complete, however, as mangoes and guava, for example, still show up to 50 per cent infestation if not sprayed, but this compares with nearly 100 per cent infestation previously. It facilitates commercial fruit production with what might be termed a normal spray program, which was difficult previously because of the enormous reservoir of flies produced in wild guava.

DACUS SPP. IN OTHER COUNTRIES.—Attempts at biological control of fruit flies have been made in many countries in addition to Hawaii. During 1909 to 1937 Silvestri imported five species of parasites and predators of the Mediterranean fruit fly from India, Africa, Brazil, and Hawaii, into Italy (Silvestri 1939), but none became established. Since the work in Hawaii in 1912 to 1914 became known, other countries in which the pest occurs, including Egypt, Spain, Israel, Algeria, South Africa, Canary Islands, Bermuda, and Brazil have attempted to utilize the same parasites in controlling it. *Opius tryoni* has not become established in any country other than Hawaii, *O. humilis* only in Bermuda, and *Tetrastichus giffardianus* only in Egypt and Brazil. The lack of success with the Opiine parasites may be attributed mainly to small releases and to the difficulties encountered in rearing them in sufficient numbers to provide adequate colonies for field release.

The olive fruit fly, *Dacus oleae* (Gmel.), a destructive pest in the Mediterranean area and the Near East, engaged the attention of Silvestri from 1910 to 1918 (Silvestri 1939), during which period he imported 15 species of parasites from various parts of Africa, mainly Eritrea, into Italy. Only *Opius concolor* Silv. from Tripoli became established, but is ineffective and of limited distribution. Several of the parasites of the oriental fruit fly have recently been released against this pest in Greece and Morocco.

Rather extensive efforts have been made to control the Queensland fruit fly, *Dacus tryoni* (Frogg.) and the Mediterranean fruit fly in Australia. The first importations were of *Syntomosphyrum indicum* from India in 1904–07, several species of *Opius* from India and Brazil in 1905–07, and a staphylinid predator from Brazil in 1905. These were followed by the three species of *Opius* from the Mediterranean fruit fly in Hawaii in 1932–33, four species of the same genus from India in 1935–36, and finally four of the most promising of the *Opius* parasites of the oriental fruit fly from Hawaii in 1952–53. None of the 17 or more species imported during the 50-year period have been reported as being established.

Since its establishment in Hawaii in 1916 the melon fly parasite, *Opius fletcheri*, has been imported into Australia, the Philippine Islands, Guam and the Ryukyu Islands, and has been reported as established in all of these except Australia. Additional importations into Guam have been *Opius watersi* Full., originally from India on the same host, and several others in 1950, but none of these persisted in the field.

In addition to the work on fruit flies that has already been mentioned, small scale attempts have been made on other species of the genus *Dacus*. In Fiji three of the Mediterranean fruit fly parasites from Hawaii were colonized in 1935 against *Dacus passiflorae* Frogg., followed by two pupal parasites from India and Australia in 1937–38, and by *Opius oophilus* from Hawaii in 1951. Only the pupal parasite, *Tetrastichus giffardianus*, became established. Three pupal parasites from Fiji and Australia were released against the same pest in Samoa during 1935–38, of which *Syntomosphyrum indicum* Silv. became established. Releases of Mediterranean fruit fly parasites have also been made against *Dacus melanotus* Coq. in the Cook Islands in 1936–38, but without success, and of two pupal parasites, *Dirhinus auratus* Ashm. and *Spalangia* sp., from Malaya on *Dacus* spp. in 1936 in Ceylon. In the latter instance, both species are reported to be established. Three species, including the above two pupal parasites were colonized upon *D. ciliatus* Loew and *D. emmerezi* Bezzi in Mauritius in 1933–34 and 1939, but have not become established.

ANASTREPHA IN CENTRAL AND SOUTH AMERICA.—Efforts have been made to utilize the parasites of the Mediterranean fruit fly and the oriental fruit fly in control of various species of the genus *Anastrepha* in South and Central America and in the West Indies, where they are the dominant fruit pests. During 1935–37, six of the fruit fly parasites from Hawaii, one from West Africa and 11 obtained from other species of *Anastrepha* in South and Central America, were introduced into Puerto Rico against the West Indian fruit fly, *Anastrepha mombinpraeoptans* Sein, and others (Bartlett 1941). Only the pupal parasite, *Spalangia philippinensis* Full. became established. Likewise, only *Tetrastichus giffardianus* became established in Brazil. A number of the oriental fruit fly parasites were colonized on *Anastrepha* in Mexico and Bolivia during 1953–54, but the outcome is not yet known, though recoveries of *O. longicaudatus novocaledonicus* have been made in Mexico. *Eucoila pelleranoi* Bréthes from Argentina is reported to have been established on *Anastrepha* in Peru.

RHAGOLETIS IN NORTH AMERICA.—Several efforts in

biological control of fruit flies of the genus *Rhagoletis* have been made in the United States, but thus far none of the parasites have become established. In the 1930's three parasites of the Mediterranean fly from Hawaii and *Opius melleus* Gahan from the apple maggot (*R. pomonella* (Walsh)) in the eastern states, were released against the walnut husk fly (*R. completa* Cress.) in California, followed by *Opius formosanus* in 1951–52. Large releases of four *Opius* species from the oriental fruit fly in Hawaii were made against the cherry fruit flies (*R. cingulata* (Loew.) and *R. fausta* (O. S.)) in the Pacific Northwest during 1952–53.

CAUSES OF FAILURE OF INTRODUCED OPIUS SPP.—A review of the many efforts to establish fruit fly parasites in countries other than Hawaii reveals a number of instances of success of establishment of the polyphagous pupal parasites, but, with few exceptions, a consistent failure with the more important larval parasites of the genus *Opius*. This outcome is attributed to two main causes. First, the majority of releases consisted of only the relatively small number comprising the original shipments, and these females may have been of low vitality and not mated. The rearing and mating of many *Opius* species is difficult, but insectary production or multiple shipments of large numbers of mated females appear to be essential to provide satisfactory stocks for field colonization.

The second cause of lack of success is that in many instances the parasite species used were not adapted to the hosts against which they were released. The species utilized in the great majority of cases were those originating in tropical countries that had been established in Hawaii on the Mediterranean fruit fly, the melon fly, and the oriental fruit fly. Outside of Hawaii they were widely colonized, not only upon *Ceratitis* and other species of *Dacus*, but upon *Anastrepha* and *Rhagoletis* as well. Some species of *Opius* have a wide range of hosts, all usually of the same genus, but others, such as *O. fletcheri* on the melon fly, appear to be restricted to a single host species. It seems extremely doubtful that a species of tropical or subtropical origin that normally develops in a host having several generations each year will be able to adapt itself to a single-brooded host, such as *Rhagoletis*, in a temperate area. Tests that have been made indicate that such parasites developing in these hosts emerge very early in the spring, several months before suitable host stages are available for attack. The hosts may be physiologically suitable but the life cycles are not synchronized.

HOSTS OF THE GENUS OPIUS.—In this connection, consideration may be given to the origin of the species of *Opius* most successfully utilized against the fruit flies in Hawaii. Of those established on the Mediterranean fruit fly, the most effective one, *O. tryoni*, came from *Dacus tryoni* in Australia, *O. fullawayi* from species of *Ceratitis* other than *capitata* in West Africa, and only *O. humilis* from the Mediterranean fruit fly itself in South Africa. The wide host range of *Opius tryoni* is demonstrated by the recent discovery of its heavy attack on a gall-making tephritid fly, *Procecidochares utilis* Stone in Hawaii (Dodd 1953). This fly was introduced from Mexico in 1945 for control of a weed, *Eupatorium glandulosum* HBK. The occurrence of this parasite on three fruit fly genera indicates its possible usefulness against other fruit pests of this family.

Recent tests in Mexico appear to indicate that *O. longicaudatus* var. *novocaledonicus* from the oriental fruit fly is well adapted to *Anastrepha ludens* (Loew.). On the other hand, the melon fly parasite, *O. fletcheri*, came from the same host species in India and the species of the genus that have shown the greatest effectiveness against the oriental fruit fly in Hawaii all came from the same host in Malaya, though several of them were found in India also.

From the point of view of practical results in biological control, that attained upon the oriental fruit fly was solely by parasites from the same host obtained in its native home, the Malayan and Indian regions. Parasites from all other fruit flies from many countries in the tropical and subtropical areas of the world contributed nothing. This outcome, of course, could not have been foreseen, since it is in marked contrast to that just mentioned on the Mediterranean fruit fly. The work on the latter species would seem to justify the extensive tests that have been made in many countries upon many other fruit fly species, and it gives some support to the belief that the general lack of success has been due in part, at least, to lack of proper rearing and colonization programs rather than to the unadaptability of the parasites to the pest species involved.

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The Transformation of Counts of European Chafer Larvae for Analysis of Variance[1]

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In experiments on the control of insect populations in the field, and in studies of insect ecology, experimental plot designs are frequently planned so that an analysis of variance test may be applied to the data collected.

It is characteristic of the analysis of variance that a single error is assembled from all parts of the experiment and used to discriminate between the means of treatments, or other factors, whose effects are being tested. Because the general experimental error is applied in this way, the analysis of variance assumes that the variance of the variate is unaffected by changes in the mean level of the variate (Bartlett 1947); that is, the variance is independent of the mean.

In most cases, counts of insects are distributed in such a fashion that the magnitude of the variance of a sample is dependent upon the size of the mean; if the data are to be tested with an analysis of variance, which assumes a constant variance, then the counts must be transformed to values such that the variance is independent of the mean.

Table 1.—The mean and variance of counts of European chafer larvae in permanent pasture sod, and the expected value of each variance for the linear and curvilinear regression of the variance on the mean.[a] Plot A, Irving DeCann farm, Ontario county, New York. 1951.

Observed Mean (Larvae per Unit)	Observed Variance	Expected Value of Variance for Regression Line	
		Linear	Curvilinear[b]
1.92	3.58	2.36	2.55
2.08	2.24		2.73
2.48	1.68		3.16
3.32	3.73		4.09
3.26	3.32		4.13
3.60	4.33		4.40
4.12	7.03		5.00
4.20	3.83		5.09
4.48	7.01		5.42
4.88	4.86		5.89
5.20	11.92		6.27
5.20	6.33		6.27
5.72	7.39		6.90
6.20	10.08	7.66	7.49
6.56	4.09		7.93
6.60	2.08		7.98
6.92	5.99		8.39
7.16	13.81		8.70
7.32	8.48		8.90
7.60	10.58		9.26
7.64	6.49		9.31
7.76	10.19		9.47
8.32	12.39		10.20
9.36	10.82		11.60
9.72	13.29	12.03	12.09

Linear regression coefficient $a = -0.02$, $b = 1.24$
Curvilinear regression coefficient $a_2 = 0.58$, $b_2 = 0.99$, $c = 0.02$

[a] Each observed mean and variance was calculated from 25 counts of larvae, in foot-square sampling units of soil, from a 5 ft. by 5 ft. subdivisions of a 25 ft. by 25 ft. plot.
[b] Departure from linearity not significant at .05 level.

Table 2.—The mean and variance of counts of European chafer larvae in permanent pasture sod, and the expected value of each variance for the linear and curvilinear regression of the variance on the mean.[a] Plot C, Irving DeCann farm, Ontario county, New York. 1951.

Observed Mean (Larvae per Unit)	Observed Variance	Expected Value of Variance for Regression Line	
		Linear	Curvilinear[b]
3.04	3.32	3.35	2.47
3.88	3.20		3.93
4.04	3.56		4.19
4.60	4.64		5.03
4.92	5.99		5.47
4.96	8.52		5.52
5.08	4.55		5.68
5.16	6.99		5.78
5.40	5.02		6.07
5.40	7.28		6.07
5.44	6.01		6.12
5.48	2.97		6.17
5.56	5.21	5.99	6.26
5.60	4.64		6.31
5.76	7.46		6.49
5.76	5.78		6.49
5.84	4.53		6.57
6.16	8.53		6.90
6.60	6.56		7.31
6.68	9.50		7.38
6.78	5.86		7.46
6.80	4.48		7.48
7.12	14.93		7.73
7.20	6.32		7.78
8.88	7.06	9.45	8.55

Linear regression coefficient $a = 0.18$, $b = 1.04$
Curvilinear regression coefficient $a_2 = -4.47$, $b_2 = 2.70$, $c = -0.14$

[a] Each observed mean and variance was calculated from 25 counts of larvae, in foot-square sampling units of soil, from a 5 ft. by 5 ft. subdivision of a 25 ft. by 25 ft. plot.
[b] Departure from linearity not significant at .05 level.

Cochran (1938) observed that a suitable transformation for analyses of population values may be suggested by the relationship between the mean and variance. Wadley (1950) has pointed out that, while the square root transformation is suitable for Poisson data, the logarithm of $(n+1)$ is better for the divergent distribution usually found. Bartlett (1946) stated that, above a mean of 10, transformation to $\sqrt{x}$ may be considered, from 10 to two or three, $\sqrt{x+\frac{1}{2}}$ is preferable, where x is equal to the observed count. Bartlett assumed that the relationship between the mean and the variance was a linear one, that is $\sigma^2 = KM$, where K is a constant. Upholt (1942) pointed out that the square-root transformation, $\sqrt{x}$, when applied to contagious distributions of the type which might reasonably be expected occasionally in

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Table 3.—The mean and variance of counts of European chafer larvae in permanent pasture sod, and the expected value of each variance for the linear and curvilinear regression of the variance on the mean.[a] Plot D, Irving DeCann farm, Ontario county, New York. 1951.

Observed Mean (Larvae per Unit)	Observed Variance	Expected Value of Variance for Regression Line	
		Linear	Curvilinear[b]
0.20	0.32	.16	.21
0.24	0.26		.25
0.36	0.55		.38
0.36	0.39		.38
0.36	0.39		.38
0.40	0.32		.43
0.40	0.48		.43
0.48	0.33		.52
0.48	0.57		.52
0.76	0.66		.86
0.80	0.96		.91
0.80	0.56		.91
0.84	1.23	.99	.96
0.88	0.83		1.02
0.92	0.87		1.07
0.96	1.24		1.12
1.04	1.16		1.23
1.16	1.17		1.41
1.20	1.04		1.46
1.20	1.84		1.46
1.24	1.54		1.52
1.28	2.20		1.58
1.32	2.30		1.64
1.56	1.45	1.93	2.02

Linear regression coefficient $a=-0.10$, $b=1.31$
Curvilinear regression coefficient $a_2=0.002$, $b_2=0.97$, $c=0.20$

[a] Each observed mean and variance was calculated from 25 counts of larvae in foot-square sampling units of soil, from a 5 ft. by 5 ft. subdivision of a 25 ft. by 25 ft. plot.
[b] Departure from linearity not significant at .05 level.

entomological research, introduces a definite bias into the means. He contended that the bias may be reduced, in certain cases at least, by adding one-half to each figure before extracting the square-root, so that the transformed value became equal to $\sqrt{x+\frac{1}{2}}$. Under reasonable conditions the bias introduced by the latter transformation is not sufficiently great to invalidate the analysis of variance.

Beall (1942) proposed that, generally in field data, the relationship between the mean and the variance is not a linear one as Bartlett had supposed, but a curvilinear one, where the departure of the variance from the mean becomes disproportionately great as the mean increases. On the basis of data obtained from seven field experiments on the control of insects, Beall proposed the transformation $k^{-1/2} \sinh^{-1} (kx)^{1/2}$, where k is a constant and x is the observation. The transformation gave good results, and from the analysis of the transformed data the results were found to differ markedly from those which would have been obtained from untransformed data.

This study was undertaken to determine the relationship between the mean and variance of counts of European chafer, *Amphimallon majalis* Raz., larvae in permanent pasture experimental plots, and to determine the effect of a square-root transformation of the counts upon this relationship.

METHOD.—Each of three permanent pasture sod plots, A, C, and D, 25 feet by 25 feet, on the farm of Irving DeCann, Ontario county, New York, was subdivided into areas 5 feet by 5 feet (Burrage & Gyrisco 1954). In each subdivision of each plot, the 25 foot-square units of soil were examined for European chafer larvae in the spring of 1951.

The mean and the variance of the counts in each subdivision, and the linear and curvilinear regression of the variance on the mean, were calculated for the series in each of plots A, C, and D (Tables 1, 2, and 3, respectively). The linear and curvilinear regression lines for plot D are shown in figure 1. The expected variance for each mean value was calculated from $Y=a+bx$ for each linear regression, and $Y=a+bx+cx^2$ for each curvilinear regression. In each series the linear regression coefficient was significant at the .05 level, and the sum of squares of deviations of the variance was not significantly reduced by going from linear to curvilinear regression.

If the relationship between the mean and variance of counts of insects is a linear one, the square-root transformation may be satisfactorily applied to make the variance independent of the mean (Beall 1942). The mean and variance, of the 25 counts of larvae in each subdivision of each of plots A, C, and D, were calculated for the counts transformed to $\sqrt{x+\frac{1}{2}}$, where x is the value of the observed count. The mean, variance, and correlation coefficients for the mean and variance, of observed and

Table 4.—The mean and variance of groups of 25 counts of European chafer larvae in foot-square sampling units of soil.[a] Plot A, Irving DeCann Farm, Ontario county, New York, May, 1951.

Block (25 Ft.-Sq. Units)	Original Counts (x)		Transformed Values ($\sqrt{x+\frac{1}{2}}$)	
	Mean (per Unit)	Variance	Mean (per Unit)	Variance
1	7.32	8.48	2.71	0.51
2	8.32	12.39	2.81	.10
3	6.60	2.08	2.60	.35
4	4.88	4.86	2.24	.38
5	5.20	6.33	2.32	.33
6	4.12	7.03	2.04	.46
7	3.60	4.33	1.94	.37
8	6.92	5.99	2.60	.36
9	7.76	10.19	2.81	.39
10	7.60	10.58	2.83	.29
11	9.36	10.82	3.08	.42
12	9.72	13.29	3.13	.45
13	7.64	6.49	2.79	.36
14	5.72	7.38	2.45	.24
15	6.50	4.09	2.64	.07
16	3.32	3.73	1.84	.45
17	4.48	7.01	2.14	.42
18	7.16	13.81	2.65	.69
19	6.20	10.08	2.46	.65
20	5.20	11.92	2.24	.69
21	1.92	3.58	1.42	.42
22	2.08	2.24	1.53	.24
23	3.36	3.32	1.90	.27
24	4.20	3.83	2.07	.42
25	2.48	1.68	1.69	.14
	$r=0.74$[b]		$r=0.0045$	

[a] The mean and variance were computed from the original values of the counts, and from transformed values equal to $\sqrt{x+\frac{1}{2}}$, where x is the original value of the count.
[b] Correlation coefficient significant at .01 level.

Table 5.—The mean and variance of groups of 25 counts of European chafer larvae in foot-square sampling units of soil.[a] Plot C, Irving DeCann farm, Ontario county, New York, May, 1951.

Block (25 Ft.-Sq. Units)	Original Counts (x)		Transformed Values ($\sqrt{x+\frac{1}{2}}$)	
	Mean (per Unit)	Variance	Mean (per Unit)	Variance
1	4.92	5.99	2.20	0.54
2	6.60	6.56	2.60	.32
3	8.88	7.06	2.85	1.27
4	7.20	6.32	2.73	.26
5	5.40	7.28	2.33	.46
6	5.84	4.53	2.46	.31
7	6.68	9.50	2.60	.40
8	5.76	5.78	2.44	.33
9	5.48	2.97	2.39	.26
10	4.04	3.56	2.08	.23
11	3.88	3.30	2.04	.24
12	4.60	4.64	2.19	.30
13	5.44	6.01	2.37	.32
14	5.60	4.64	2.41	.28
15	5.40	5.04	2.36	.31
16	5.08	4.55	2.31	.25
17	6.80	4.48	2.65	.29
18	5.56	5.21	2.39	.34
19	5.16	6.69	2.29	.41
20	3.04	3.32	1.81	.27
21	4.96	8.52	2.25	.41
22	6.16	8.53	2.48	.49
23	6.76	5.86	2.63	.33
24	7.12	14.93	2.67	.54
25	5.76	7.46	2.44	.33
	r=0.50[b]		r=0.20	

[a] The mean and variance were computed from the original values of the counts, and from the transformed values.
[b] Correlation coefficient significant at .01 level.

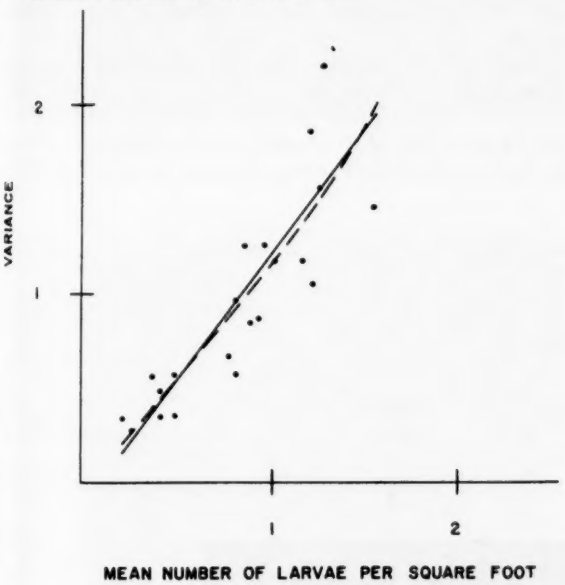

FIG. 1.—The linear and curvilinear regression of the variance on the mean, of counts of European chafer larvae in foot-square sampling units of soil in pasture sod. The solid and broken lines are the calculated linear and curvilinear regression lines, respectively. Each observed mean and its variance was determined from 25 counts of larvae, each from a foot-square unit of soil, from a 5 ft. by 5 ft. subdivision of a 25 ft. by 25 ft. plot. Plot D, Irving DeCann farm, Ontario county, New York. 1951.

transformed counts from plots A, C, and D, are shown in tables 4, 5, and 6, respectively.

DISCUSSION.—In each of plots A, C, and D, the magnitude of the variance of counts of European chafer larvae was dependent upon the size of the mean, in a linear relationship as, in each case, the coefficient of linear regression of the variance on the mean was significant, and the sum of squares of deviations of the variance was not significantly reduced by going from linear to curvilinear regression (Tables 1, 2, and 3). The correlation coefficient between the mean and variance of each group of 25 counts was not significant when each count was transformed to $\sqrt{x+\frac{1}{2}}$, where x is the observed value of the count, in each of plots A, C, and D (Tables 4, 5, and 6).

The data indicate that, where analyses of variance are to be applied to counts of larvae within the observed range of population density, the square-root transformation may be satisfactorily applied to equal the expectation of variance for all samples of 25 counts.

SUMMARY.—The mean and variance of each of 25 groups of counts of third instar European chafer larvae, each group containing 25 counts of larvae in foot-square sampling units of soil, were calculated for three different locations. The data from the calculations indicated that the variance of the counts of larvae was dependent upon the mean in a linear relationship. When the value of each count was transformed to $\sqrt{x+\frac{1}{2}}$ there was no significant correlation between the mean and the variance of the samples.

Table 6.—The mean and variance of groups of 25 counts of European chafer larvae in foot-square sampling units of soil.[a] Plot D, Irving DeCann farm, Ontario county, New York. May, 1951.

Block (25 Ft.-Sq. Units)	Original Counts (x)		Transformed Values ($\sqrt{x+\frac{1}{2}}$)	
	Mean (per Unit)	Variance	Mean (per Unit)	Variance
1	0.88	0.82	1.10	0.16
2	1.16	1.17	1.21	.19
3	1.28	2.20	1.22	.28
4	1.24	1.54	1.23	.23
5	1.56	1.45	1.36	.20
6	0.92	0.87	1.03	.36
7	1.20	1.04	1.00	.69
8	0.48	0.57	0.93	.12
9	0.24	0.26	0.82	.07
10	0.36	0.39	0.87	.10
11	0.40	0.48	0.89	.11
12	0.76	0.66	1.06	.14
13	1.04	1.16	1.16	.20
14	1.32	2.30	1.23	.31
15	0.40	0.32	0.90	.10
16	0.80	0.96	1.06	.18
17	0.84	1.22	1.08	.20
18	0.36	0.39	0.87	.10
19	0.20	0.32	0.79	.07
20	0.48	0.33	0.94	.10
21	0.36	0.55	0.86	.11
22	1.20	1.84	1.20	.26
23	0.96	1.24	1.13	.19
24	0.80	0.56	1.08	.13
	r=0.87[b]		r=0.38	

[a] The mean and variance were computed from the original values of the counts, and from the tranformed values.
[b] Correlation coefficient significant at .01 level.

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The Feeding Mechanism of Hessian Fly Larvae[1,2,3]

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The feeding mechanism of the hessian fly (*Phytophaga destructor* Say) larvae is one of the unsolved problems in the life history of the insect. No damage can be observed on the plant stem under the head or body of the larva, and there is only speculation concerning the method of feeding. Marchal (1897) stated that the larva fixes its head at the node of the plant stem and begins to nourish itself. It remains immobile, once it is established, and sucks the nourishing juices from the plant until it has attained its definitive size. Haseman (1930) also concluded that the larvae are sap feeders. Lying as it does, between the base of the leaf sheath and the stem of the wheat plant, the larva firmly attaches its mouth parts to the surface layer of the plant cells and extracts its food.

The present study was a part of a broad investigation of biochemical factors involved in the resistance of wheat plants to attack by hessian flies (Refai *et al.* 1955). The objective was to determine the way larvae obtain food, in order to understand the physiological damage that occurs in infested wheat plants.

MATERIALS AND METHODS.—Active young larvae, 2 to 5 days old, were obtained from cultures maintained by the Entomology Research Branch Hessian Fy Laboratory at Manhattan, Kansas. Plants infested with larvae of the desired age were trimmed to remove part of the leaves and the roots. The stems that remained and the attached larvae were then placed either in a glass containing a small amount of water or wrapped first in moist paper toweling and then in wax paper. This material was stored in a refrigerator (at 10° C.) until needed. The cold infested wheat stems were removed from the refrigerator approximately 3 to 4 hours before observing feeding action. The cold larvae were inactive.

An electronically amplified listening device (Adams *et al.* 1953, 1954) was used to hear the sounds produced by the feeding young larva. The sounds were recorded by using a combination of the listening device, an oscilloscope and a Polaroid Land camera (Adams *et al.* 1953).

A moving picture sequence of feeding larvae was taken at the Photographic Productions Laboratory, University of Nebraska. A microscope fitted with accessories for motion photography[4] was used. The specimen was illuminated with a Zirconarc lamp.[5] Pictures were taken with an Eastman Cine Kodak Special 16 mm. movie camera.

RESULTS AND DISCUSSION.—An intermittent pulsing noise made by one or more feeding larvae was demonstrated by using the electronically amplified listening device. A recording of this sound on an oscilloscope screen is shown in figure 1. The sound was a pulsation of short

[1] Cooperative investigations between the Field Crops Research Branch and the Entomology Research Branch, Agr. Res. Service, U.S.D.A., and the Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. Contribution No. 260, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. This work was supported in part by a grant from the National Science Foundation. Accepted for publication September 1, 1955.

[2] A portion of the work presented here was included in a dissertation submitted by Fawzy Y. Refai to the Graduate School, Kansas State College, in partial fulfillment of the requirements for the degree, Doctor of Philosophy, May 1955.

[3] Presented at the Third Annual Meeting of the Entomological Society of America, Cincinnati, Ohio, November 28–December 1, 1955.

[4] Bausch & Lomb Optical Co., Rochester, 2, N. Y.

[5] Fish-Schurman Corp., 70 Portman Road, New Rochelle, N. Y.

FIG. 1.—Oscilloscope pattern of sound made by a feeding hessian fly larva.

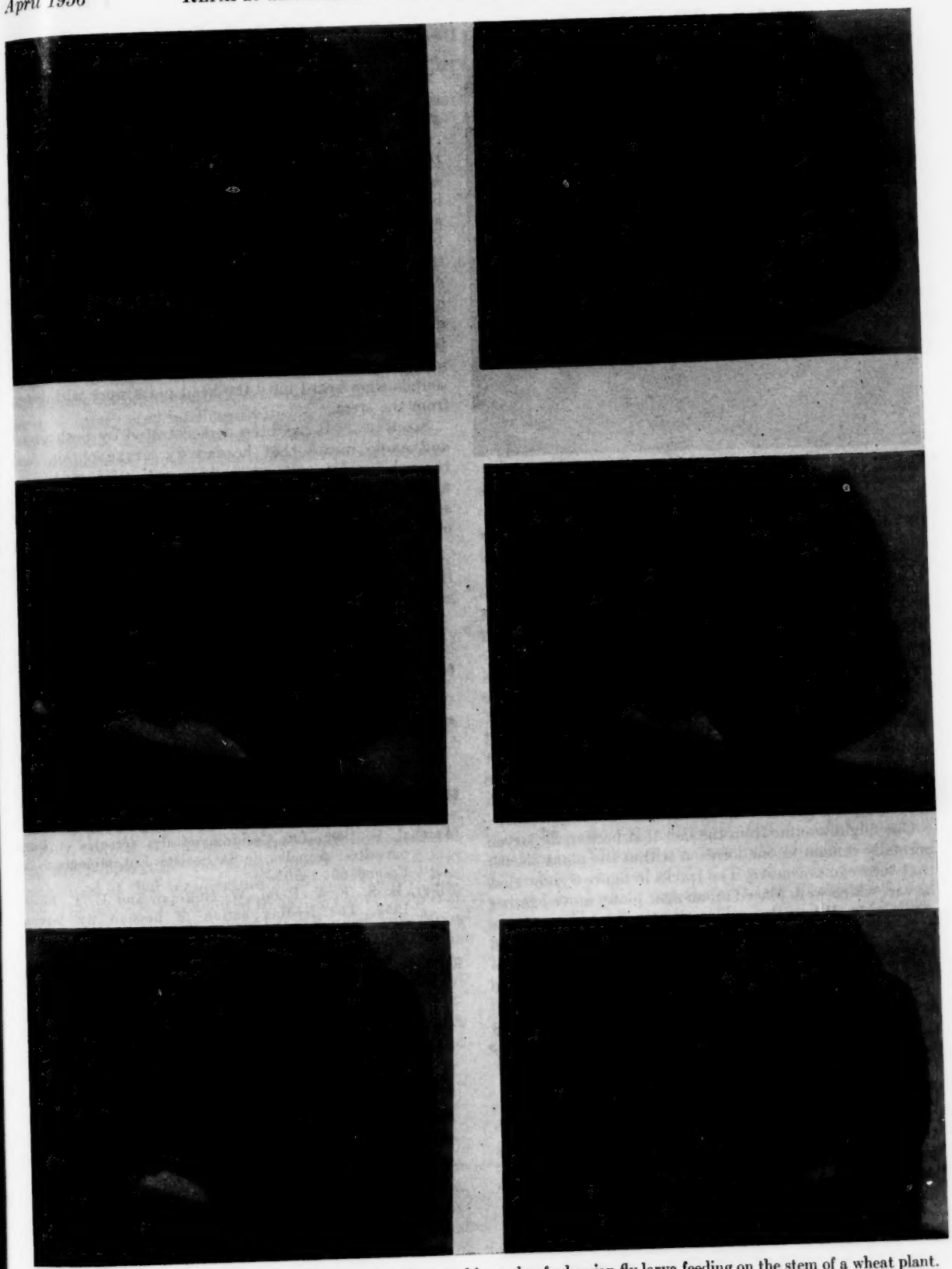

FIG. 2.—A selected sequence of frames from a cinémicrographic study of a hessian fly larva feeding on the stem of a wheat plant.

FIG. 3.—Tracks indicating movement of three day old hessian fly larvae on an agar plate.

duration. This pulse of sound is superimposed over the bright spot at the left end of the picture (Fig. 1). No pulses resulting from the circulatory or respiratory systems of non-feeding larvae were observed.

Microscopic observation of a feeding larva showed that the head of the larva approached the wheat plant stem in an intermittent fashion. This approach was followed by the movement of the invaginating part of the larva's body which formed a cup-like structure around the mouth parts and made complete contact with the plant stem. The larva stayed in this position for a while and then removed its entire buccal extremity from the plant stem. This sequence was recorded in a cinémicrographic study (Miller *et al.* 1955). Selected frames of this action are shown in figure 2.

One might assume from the fact that hessian fly larvae normally remain in one location within the plant sheath that they can not move. The tracks in figure 3 show that larvae, which were placed on an agar plate, moved rather extensively. When observed under the microscope, these larvae appeared to be searching for food. It is presumed that the larvae fixed to the wheat plant stem do not move because their food, consisting of plant juice, moves up to them through the stem of the wheat plant.

To associate the moving action of the mouth parts with the intermittent feeding sounds, the hearing aid microphone was removed from the sound-proof box of the listening device and placed on the stage of a microscope. A variable Band-Pass Filter[6] was used to exclude extraneous sounds. A frequency range of sounds due to feeding of the larvae ranged from 300 to 2000 cps. This work was carried on at night to eliminate noises not associated with the insects. The infested stem was inserted first in the sound insulated and electrically shielded chamber of the listening device to make sure that the larvae under examination were feeding. The sheath was then stripped from the stem and the actions of the different larvae on the stem were observed until an active one was located. All but one active larva were removed. This larva was observed by using both the microscope and the listening device. In more than one case, the tested larva was seen to move its mouth parts towards the plant until it came in close contact with the stem. Intermittent sucking sounds were heard until the head parts were withdrawn from the stem.

SUMMARY.—It has been demonstrated by both visual and audio means that hessian fly larvae obtain food through an intermittent sucking action. A cinémicrographic study of this sucking action was made. Similar sound and motion studies might be useful in studies of other insects.

ACKNOWLEDGMENTS.—The technical assistance of Wendell Hoffman, photographer, University of Nebraska at Lincoln, is gratefully acknowledged.

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[6] Krohn-Hite Instrument Co., Cambridge, Mass.

Fate of P^{32}-Labeled Malathion in the Laying Hen, White Mouse, and American Cockroach[1]

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Recent investigations (Vincent *et al.* 1954, Furman *et al.* 1955) indicate that malathion [*0,0*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorodithioate] is an especially valuable insecticide and acaricide for controlling ectoparasites of poultry and that it may be conveniently applied by dusting or spraying the birds themselves or the litter. The experiments described below were undertaken in order to assess the extent of possible contamination of meat and eggs of treated birds, and to obtain information on the rate of elimination and metabolism of malathion in warm-blooded animals and insects, factors which must be considered if the toxicology of any residues are to be interpreted intelligently.

MATERIALS AND METHODS.—*Tracer Preparation.*—The synthesis of the P^{32}-labeled malathion was accomplished according to the following equations:

$$2P^{32}+5S\rightarrow P^{32}_2S_5$$

$$P^{32}_2S_5+4CH_3OH\rightarrow 2(CH_3O)_2P^{32}SSH+H_2S$$

$$(CH_3O)_2P^{32}SSH+\underset{\underset{CH-COOEt}{\|}}{CH-COOEt}\rightarrow(CH_3O)_2P^{32}(=S)-S-\underset{\underset{CH_2COOEt}{|}}{CH-COOEt}$$

Red phosphorus (3.2 g. Baker and Adamson's amorphous red powder) was sealed in a quartz ampule and irradiated at Oak Ridge, Tennessee, for 4 weeks in the neutron pile. The calculated activity of the radioactive phosphorus was approximately 200 mc.

In a 50-ml. three-necked flask equipped with stirrer, dropping funnel, and condenser, were placed 10.2 g. sulfur, 1.74 g. phosphorus pentasulfide, and 3.2 g. red phosphorus (P^{32}). Dry nitrogen was passed through the system, and the flask was heated in a silicone bath until the mixture melted. It was then stirred until homogeneous, and the temperature was slowly raised to 280° C. and maintained at that temperature for 5 hours without stirring. The mixture was allowed to cool to 80° C. ,15 ml. of anhydrous toluene was added, and 8 g. of anhydrous methanol was added dropwise over a 1-hour period. The temperature was maintained at 80°–90° C. until the evolution of hydrogen sulfide ceased. The solution was filtered into a 100-ml. three-necked flask equipped with a condenser (vacuum attached) and a dropping funnel. A stirrer was then attached, the mixture was warmed to 50°–55° C., 10.2 g. diethyl maleate was added in one portion, and the toluene was removed under reduced pressure. The temperature was raised to 85° C. and the mixture was stirred for 5 hours, after which it was allowed to cool to room temperature, washed with 20 ml. of 10% sodium carbonate in water, and stripped under 0.3-mm. pressure. The yield of P^{32}-labeled malathion was 15 g., with an initial relative activity of 11.1 c.p.s./γ. Examination of the product by paper chromatography showed that the radioactive portion was 94% malathion. A portion of the product was washed further with 10% sodium carbonate and water, and a product of 99% purity was obtained.

The partition of the malathion between chloroform and water was determined by dissolving 1.7 mg. of P^{32}-labeled malathion in 100 ml. of water and extracting with an equal volume of chloroform. The first extraction showed a chloroform:water-partition ratio of 70:1 (average). The second extraction showed a chloroform:water-partition ratio of 0.13:1. It is apparent, therefore, that essentially all of the malathion in water is taken up by chloroform after one extraction.

Preparation of Possible Metabolites.—0,0 Dimethyl hydrogen phosphorodithioate.—In a 200-ml. three-necked flask equipped with condenser, stirrer, and dropping funnel, was placed 40 ml. of anhydrous benzene. The stirrer was started, and 35.2 g. of phosphorus pentasulfide was slurried in. The temperature of the mixture was maintained at 40° C., and 28.8 ml. of anhydrous methanol was added dropwise. Stirring was continued for 3 hours, and the solvent was removed under water aspirator pressure. The phosphorodithioic acid was then distilled; b.p. 42°–44° C. (0.5 mm.), yield 35 g.

0,0-Dimethyl *S*-(1,2-bis-carboxy)-ethyl phosphorodithioate.—A mixture of 15.8 g. of *0,0*-dimethyl hydrogen phosphorodithioate and 9.8 g. of maleic anhydride was heated at 85° C. for 15 hours. A light-reddish oil was obtained, to which 6 g. of water was added; the mixture was then heated until solution occurred. Benzene was added, and the excess water was removed by azeotroping the mixture. The benzene was removed under vacuum, and the oil obtained was induced to crystallize by scratching. The product was recrystallized twice from chloroform, m.p. 115°–118° C. Calculated for $C_6H_{11}O_4PS_2$; N.E., 135; P, 11.3. Found: N.E., 137; P, 12.0.

0,0-Dimethyl *S*-(1-carboxy-2-carboethoxy)-ethyl phosphorodithioate.—A mixture of 14.4 g. ethyl hydrogen maleate and 15.8 g. of *0,0*-dimethyl hydrogen phosphorodithioate was heated at 85° C. for 15 hours. A viscous oil was obtained which was heated to 80° C. under 0.3-mm. pressure. The oil could not be induced to crystallize, $n_D^{24}=1.5149$. Calculated for $C_8H_{15}O_4PS_2$; N.E., 302.3; P, 10.2. Found: N.E., 287; P, 10.4. In the determination of the neutral equivalent, by titrating the half-ester with standard alkali the pink color indicating the phenolphathalein end-point disappeared quite rapidly; this points to moderately fast hydrolysis of the half-ester, which probably accounts for the low N.E. value.

Experiments with Hens.—Twenty-eight 8- to 9-month-old White Leghorn laying hens (obtained from the Heisdorf & Nelson Farm, Riverside, California) were used in these experiments. They were housed singly in standard two-bird wire cages. Twenty per cent breeders' mash (obtained through the Poultrymen's Co-Operative Asso-

[1] Paper No. 877, University of California Citrus Experiment Station, Riverside, California. Part I of a series of papers on the fate of malathion in warm-blooded animals and in insects. This work was supported in part by grants from the U. S. Atomic Energy Commission, Contract AT(11-1) 34, Project 6, and from the American Cyanamid Company, New York, N. Y. Accepted for publication September 6, 1955.

ciation of Southern California) was fed at the maximum amounts the birds would eat.

Droppings.—Droppings were individually collected in tared aluminum pans formed from freezer-weight aluminum foil. Droppings collected over 1- to 4-day intervals were weighed, thoroughly mixed, and a subsample of about 5 g. was removed to the laboratory. A second subsample of approximately 500 mg. was weighed to the nearest 0.1 mg., homogenized in 3 ml. of water in an all-glass Potter-Elvejhem homogenizer, shaken with 3 ml. of chloroform, and centrifuged for 10 minutes at 25,000 times gravity to separate the two phases and the insoluble residue. One- to 2-ml. aliquots of the two phases were taken for radioassay. The aliquots were placed in stainless-steel cupped counting planchets, 1 inch in diameter and 5/16-inch deep, and were evaporated under a gentle stream of air and counted by means of a standard Geiger-Müller thin end-window tube. Quantitative values, after correction for dilution, background, coincidence loss, and progressive decay, were obtained by comparison with radioactive malathion standards.

Tissues.—The hens in preparation for sacrificing were anesthetized with chloroform and weighed, and the breast and legs were plucked. The thorax was opened, and a sample of blood was drawn from the heart into a citrated syringe. The breast, drumsticks, liver, gizzard, and kidneys were removed, and 1-g. samples of the tissues were obtained at random from a number of locations. One set of these samples was homogenized, partitioned between chloroform and water, and counted as noted above for the droppings. A second set was weighed directly into the cupped counting planchets, wet-ashed with HNO_3, muffled at 450° to 500° C., and counted for total radioactive residue. A few determinations were made by this total residue technique for brain, skin (from the breast), and feathers.

For comparative purposes relatively large amounts of breast (34 to 108 g.), drumstick (35 to 82 g.), and liver (22 to 50 g.) were homogenized with 200 ml. of chloroform in a Waring Blendor. The homogenate was filtered, washed with two additional 100-ml. aliquots of chloroform, and the filtrate evaporated nearly to dryness in a Kuderna-Danish evaporative concentrator. The residual liquid was made up to 5 ml. with chloroform, and 1- and 2-ml. aliquots were counted for total radioactivity. This technique was applied to tissues from the hens fed 100 p.p.m. of malathion, and only the chloroform-soluble residues in the tissues were extracted in this way.

Eggs.—The evaluation of the radioactive contamination in the eggs from the treated hens presented a difficult problem because of the proteinaceous nature of the substrate, and several methods were utilized to give a composite picture. In the experiments with birds fed radioactive malathion, small samples (ca. 500-1000 mg.) of yolk and white were removed by hypodermic syringe and weighed directly into the stainless-steel counting planchets. After drying, these were counted as described above. This method measured the total radioactivity present in the sample. The remainder of the egg was then homogenized in the Waring Blendor in 100 ml. of chloroform, and the chloroform layer was separated and concentrated as far as possible in the Kuderna-Danish evaporative concentrator. Duplicate 1-ml. aliquots were evaporated in planchets and counted for total radioactivity. Because of the large amount of egg-substance extracted by the chloroform, it was necessary to correct the radioactive count for self-absorption, by a factor of 1.15.

Subsequently, it was found that a very precise and convenient method for measuring total radioactivity in eggs to as little as 0.01 p.p.m. malathion, was the utilization of a Geiger-Müller liquid dip tube (D52; Nuclear Instrument and Chemical Corporation, Chicago, Illinois) immersed directly in 12.0 ml. of egg yolk or white. The dip tubes were calibrated by immersion in 12.0 ml. of egg yolk fortified with varying amounts of P^{32}-malathion to give a standard curve for each tube used. This method was utilized in evaluating the total radioactivity of eggs from the birds sprayed with 0.5% P^{32}-malathion, and the data were calculated as p.p.m. of malathion (M.W. 331).

Feeding Experiments.—One (1.000) g. of P^{32}-malathion dissolved in 500 ml. of acetone was sprayed over 10,000 g. of breeders' mash while it was being blended for 2 hours in a ribbon mill. The final mixture contained 100.6 p.p.m. by weight of P^{32}-malathion in the mash, as determined by counting four replicated samples selected at random. The radioactive feed was individually fed to 11 hens (average weight 1580 g.) in individual round aluminum pans $4\frac{1}{2}$ inches in diameter and $2\frac{1}{4}$ inches deep. Each pan was covered with a plastic disc having a hole 2 inches in diameter in the middle. The pans were tared and the amount of food consumed daily was determined for each hen. The hens were fed radioactive feed for 15 days and then the four remaining hens were placed on normal feed for 10 additional days to determine the rate of elimination of the radioactive residues. Hens were sacrificed at 4, 5, 6, 7, 11, 13, and 15 days after being on radioactive feed, and at 3, 5, 7, and 10 additional days after being placed on normal feed. The hens were individually housed in cages, and the droppings and eggs were individually collected.

Spray Experiments.—An emulsion concentrate was prepared containing 57% P^{32}-malathion, 32% xylene and 11% emulsifier (Triton X-100). A water emulsion was prepared from this concentrate by adding 4.4 g. to 500 ml. of water. The resulting water emulsion thus contained 0.5 per cent by weight of P^{32}-malathion. Twelve hens (average weight 1690 g.) were individually sprayed with 38 ml. of the water emulsion by means of a small self-contained air-pressure sprayer (Sure-Shot; Milwaukee Sprayer Mfg. Co., Inc., Milwaukee, Wisconsin). The birds were held by the tips of the wings and thoroughly wet with spray except for the area about the beak, the upper surfaces of the wings and a small part of the back between the wings. There was no appreciable runoff from the 38 ml. of spray. This application was equivalent to 1 gal. of a 0.5% water emulsion to 100 birds. The dose per bird was 190 mg. of P^{32}-malathion, which is equivalent to 112 p.p.m. for a 1690 g. bird. After spraying, the hens were individually housed in the wire cages and the droppings and eggs were individually collected. Hens were sacrificed 1, 2, 3, 4, 7, 9, 14, 18, 21, 25, 28, and 32 days after treatment.

Intraperitoneal Injections.—Four hens were injected intraperitoneally with from 0.16 to 0.25 ml. of P^{32}-malathion. Droppings and tissues from these birds were collected and prepared in the same way as noted above.

Experiments with Mice.—White mice were intraperitoneally injected with 0.1 ml. of a 5% solution of P32-malathion in propylene glycol or 0.01 ml. of undiluted P32-malathion by means of a micrometer-driven syringe (March & Metcalf 1949). The treated mice were confined in metabolism cages modified after Roth *et al.* (1948) for the separate collection of urine and feces. Metabolites excreted in the urine were examined by paper-chromatographic techniques.

Experiments with the American Cockroach.—The experiments with the adult American cockroach, *Periplaneta americana* (L.), were similar to the *in vivo* experiments previously described by March *et al.* (1955) for Systox. One to 2 μl. of undiluted P32-malathion, or of a 5% solution in propylene glycol, were applied topically on the ventral cervical membrane or injected through the mesothoracic spiracle by means of a micropipette or the micrometer-driven syringe noted above. The intact guts, nerve cords, or thoraces dissected from a number of roaches were examined singly or pooled, homogenized in water, and partitioned between chloroform and water to obtain extracts for radioassay or preparation of paper chromatograms.

Paper Chromatography.—The P32-malathion and its metabolites from chicken droppings, mouse urine, or cockroach tissues were examined by paper-chromatographic techniques similar to those previously described by March *et al.* (1954). The ascending technique of paper chromatography was employed, using tapered strips of Whatman No. 1 filter paper, 300 mm. in length, 24 mm. in width at the top, and 18 mm. in width at the bottom. The solvents which were found to be most satisfactory for separating the greatest number of components from the applied mixtures were as follows: (1) paper impregnated with 1% Silicone 550 (Dow Corning Corporation, Midland, Michigan), mobile solvent, the upper phase from a mixture of *n*-butyl alcohol 10 parts, water 10 parts, and propionic acid 1 part (by volume); and (2) paper impregnated with 4% mineral seal oil, mobile solvent, the upper phase from a mixture of *n*-butyl alcohol 10 parts, water 10 parts, acetic acid 1 part (by volume).

Other techniques.—Techniques such as those used in radioassay; radioautography; partitioning between immiscible solvents; contact toxicity to the citrus red mite, *Metatetranychus citri* (McG.), the greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché), larvae of the southern house mosquito, *Culex quinquefasciatus* Say, and the house fly, *Musca domestica* L.; and in the inhibition of house-fly cholinesterase have been described in previous publications (March & Metcalf 1949, Metcalf *et al.* 1949, Metcalf & March 1952, Pietri-Tonelli & March 1954, Metcalf *et al.* 1954, and March *et al.* 1954).

EXPERIMENTAL RESULTS.—*Feeding Experiments with Hens.*—The distribution of P32-malathion and metabolites in hen droppings after various time intervals is shown in table 1 (data based on malathion M.W. 331). By the fourth day the wet droppings contained 40 to 60 p.p.m. of total radioactive compounds, or about half the concentration being fed in the dry feed. The amount extracted from the droppings decreased very abruptly as soon as the hens were placed on normal feed, and then slowly continued to decrease. The data showing relationship of the amount consumed to that eliminated in the droppings are summarized in table 2. These data indicate that after 2 to 4 days more than 60 per cent of the consumed malathion had been eliminated and that after 5 to 6 days more than 75 per cent was eliminated daily in the droppings. This does not indicate, however, that the greater part of the malathion passed through the digestive tract unchanged. The data in table 2 for the partition of the extracted compounds between chloroform and water show that on the average only 2 to 3 per cent of the

Table 1.—P32-malathion and metabolites in hen droppings at intervals while being fed radioactive feed and after return to normal feed.[a]

	P32-MALATHION AND METABOLITES IN DROPPINGS, γ/g.											
	Hen Number											Average per Hen
DAYS	2	9	7	5	10	6	8	4	1	3	11	
	On radioactive feed (100 p.p.m. P32-malathion)											
1	0.53	8.5	0	16.9	8.5	24.3	25.2	23.5	17.1	19.1	0	13.0
4	71.5[b]	38.3[c]	75.6	49.8	58.9	44.6	51.0	43.4	33.7	43.4	40.6	50.0
6			52.7[d]	46.5[e]	49.1	44.0	63.5	63.3	47.6	47.1	42.3	50.7
8					36.8	44.8	51.6	44.6	50.5	50.8	36.6	45.1
11					37.3[f]	45.9	47.1	43.2	44.0	39.2	33.4	41.6
13						31.9[f]	39.3	42.0	35.1	44.9	52.8	41.0
15							67.8[f]	56.6	67.2	69.6	63.3	64.9
	On normal feed											
18								2.3[f]	2.5	1.1	0.88	1.7
20									1.9[f]	0.42	1.5	1.3
22										1.1[f]	0.25	.68
25											0.32[f]	.32

[a] Radioactive feed replaced by normal feed after 15 days.
[b] Hen sacrificed at 4 days.
[c] Hen sacrificed at 5 days.
[d] Hen sacrificed at 6 days.
[e] Hen sacrificed at 7 days.
[f] Hen sacrificed at longest time interval shown.

Table 2.—Relation between amount of P^{32}-malathion consumed in feed and amount of P^{32}-malathion and metabolites excreted in droppings and and average values for partition between chloroform and water of P^{32}-malathion and metabolites in droppings.[a]

Days	Ave. Consumption per Day per Hen		Ave. Excretion of P^{32}-Malathion and Metabolites in Droppings per Hen			Per Cent P^{32}-Malathion and Chloroform-Soluble Metabolites in Droppings		Per Cent Water-Soluble Metabolites in Droppings	
	Radioactive Feed, g.	P^{32}-Malathion, mg.	Mg. per Sampling Period	Mg. per Day	Per Cent per Day	Average	Range	Average	Range
				On radioactive feed (100 p.p.m. P^{32}-malathion)					
1	92	9.2	2.6	2.6	28	2	1–6	98	94–99
2–4	52	5.2	10	3.3	63	1	1–2	99	98–99
5–6	66	6.6	10	5.0	75	3	1–6	97	94–99
7–8	74	7.4	15	7.5	100	3	1–4	97	96–99
9–11	89	8.9	21	7.0	79	3	1–5	97	95–99
12–13	78	7.8	13	6.5	83	2	1–5	98	95–99
14–15	93	9.3	15	7.5	81	1	1	99	99
				On normal feed					
16–18	0	0	0.62	0.21	2.3[b]	4	0–8	96	92–100
19–20	0	0	0.31	0.16	1.7[b]	3	0–6	97	94–100
21–22	0	0	0.26	0.13	1.4[b]	16	0–32	84	78–100
23–25	0	0	0.063	0.021	0.2[b]	0	0	100	100

[a] Radioactive feed replaced by normal feed after 15 days.
[b] Based on average mg. P^{32}-malathion consumed per hen on 15th day.

consumed malathion was excreted unchanged or as chloroform-soluble metabolites. Ninety-seven to 98 per cent was excreted as ionic water-soluble metabolites and degradation products. The amount of malathion passing directly through the digestive tract, in comparison to the amount absorbed and eliminated through the kidneys, could not be readily determined. The data suggest that a considerable amount of the malathion consumed must be metabolized in the gut and excreted directly without being absorbed.

The data in table 3 show the amounts of malathion and metabolites recovered from various hen tissues during the experimental period. Although considerable amounts were absorbed, the data demonstrate that the accumulation of malathion or its metabolites in the tissues was slow and that the greatest amount found was less than 3 per cent of the concentration being fed. The blood contained a relatively constant amount of radioactive compounds during the period the hens were on radioactive feed. Of the tissues examined, liver and kidney contained the greatest quantities of radioactivity. In general, the amount of malathion and metabolites in the tissues increased slowly during the feeding period and then decreased when the hens were placed on normal feed. As in the droppings, only a small fraction of the total radioactive compounds was in the form of unchanged malathion and chloroform-soluble metabolites. The data indicate that the absorbed malathion is rapidly metabolized and eliminated through the kidneys.

The amounts of malathion and metabolites obtained from the assay of 37 eggs is shown in table 4. From these data it is clear that appreciable amounts of radioactivity appeared in the eggs shortly after the beginning of the feeding experiment and increased to a maximum level of 1 to 3 p.p.m. of total radioactivity, calculated as malathion, 1 to 2 weeks after feeding began. After removal of the malathion-fortified feed in 15 days, the level of activity appeared to remain relatively constant for another week and then slowly decreased. The amounts of radioactive compounds in the white appeared to decrease more rapidly than those in the yolk.

The total radioactivity represents the maximum amount of malathion, which may be present. However, as shown previously for droppings and tissues, malathion is readily metabolized to predominantly water-soluble substances, which comprise the major portion of the residues. The values in table 4 for the chloroform extract of whole eggs represent the malathion and chloroform-soluble metabolites and comprise, on the average, 7 per cent of the total radioactivity (as calculated from yolk values). The chloroform-soluble compounds showed a fairly regular increase from trace amounts of 0.005 p.p.m. after 2 days to a maximum of 0.10 p.p.m. at about 2 weeks, and then slowly declined when the fortified feed was withdrawn.

Spray Experiments with Hens.—The distribution of P^{32}-malathion and metabolites in the droppings of the sprayed hens after various time intervals is shown in table 5. The largest amount of absorption and excretion in any one day took place within the first 24 hours. Then both absorption and excretion gradually decreased during the remainder of the experimental period. The maximum concentration of radioactive compounds in the droppings of the sprayed hens was about one half of that found for the fed hens. The relationship of the amount applied to that excreted in the droppings is shown in table 6. About 2 per cent of the applied dose was absorbed and excreted within the first 24 hours, and after 32 days only about 12 per cent of the applied dose had been absorbed and excreted. By way of contrast, the fed hens eliminated over 75 per cent of the amount being fed daily, after 5 to 6 days The relative amounts of unchanged malathion

Table 3.—Distribution of P^{32}-malathion and metabolites in hen tissues at intervals while being fed radioactive feed and after return to normal feed.[a]

Days	Hen No.	Fraction[b]	P^{32}-Malathion and Metabolites in Tissues, γ/g.					
			Breast	Drumstick	Liver	Gizzard	Kidney	Blood[c]
On radioactive feed (100 p.p.m. P^{32}-malathion)								
4	2	T	0.19	0.21	1.35	0.58	2.08	1.30
		C	0.0056	0.023	0.089	0	0.0076	0.0
5	9	T	0.16	0.27	1.27	0.52	1.94	1.24
		C	0.0060	0.018	0.066	0	0.018	0.0
6	7	T	0.18	0.36	1.44	0.71	2.56	1.48
		C	0.0089	0.012	0.060	0	0	0.0033
7	5	T	0.22	0.41	1.56	0.65	1.94	1.02
		C	0.0093	0.021	0.074	0	0.030	0
11	10	T	0.34	0.56	2.59	0.77	2.65	1.22
		C	0.017	0.019	0.15	0.029	0.031	0.026
13	6	T	1.02	0.50	2.71	0.42	1.38	1.08
		C	0.31	0.012	0.013	0	0.071	0.020
15	8	T	1.18	0.33	2.12	0.50	0.93	0.44
		C	0.014	0.024	0.44	0.010	0	0.21
On normal feed								
18	4	T	0.33	0.34	1.33	0.19	0.53	0.56
		C	0.0072	0.018	0.027	0	0.060	0.020
20	1	T	0.24	0.18	0.41	0.11	0.21	0.089
		C	0.0024	0.0042	0.0085	0	0	0
22	3	T	0.22	0.24	0.76	0.14	0.16	0.070
		C	0.058	0.024	0.27	0	0.016	0
25	11	T	0.12	0.098	0.14	0.55	0.12	0.049
		C	0.0027	0.0027	0.013	0	0.058	0

[a] Radioactive feed replaced by normal feed after 15 days.
[b] T, maximum total residue P^{32}-malathion and metabolites; C, maximum residue P^{32}-malathion and chloroform-soluble metabolites.
[c] γ per ml. instead of per g.

and chloroform-soluble metabolites were somewhat greater in the droppings of the sprayed hens than in those of the fed hens, averaging about 10 per cent during the experimental period. Most of the excretory products, however, were in the form of water-soluble metabolites.

Considerably smaller residues of malathion and metabolites were found in the tissues of the sprayed hens, being only about one-tenth of those found in the tissues of the fed hens (see Table 7). Ten days after the fed hens were placed on normal feed, however, the residues in their tissues began to approach those found in the sprayed hens. The relative amounts of unchanged malathion and chloroform-soluble metabolites were somewhat greater in the droppings and tissues of the sprayed hens than in those of the fed hens during the first 2 weeks after spraying. After 2 weeks the residues in the tissues of the sprayed hens were entirely in the form of water-soluble metabolites. This relatively larger amount of unchanged malathion and chloroform-soluble metabolites in the droppings and tissues of the sprayed hens may probably be accounted for by the rapid removal of the absorbed malathion from the blood by the kidneys before it was completely metabolized. The malathion absorbed from the digestive tract in the fed hens had to pass through the liver before entering the general circulation for subsequent removal by the kidneys, and the high metabolic activity of the liver toward related organo-phosphorus insecticides is well known (Mazur 1946, Cheng 1951, Augustinsson & Heimbürger 1954, and March *et al.* 1955). The relatively high residues on the skin and feathers of the sprayed hens suggest that malathion is not very rapidly absorbed through the skin and that the malathion absorbed by the feathers may not be readily available for further absorption into the body. Chromatograms of acetone extracts of the feathers (see Fig. 1) showed only a single spot at the solvent front, which most likely is unchanged malathion.

The amounts of malathion and metabolites obtained from the assay of 109 eggs are shown in table 8. In these experiments, total radioactivity was determined principally in the yolk, as the levels of contamination were low and no radioactivity was detected in a few determinations of the white. In these eggs radioactivity gradually accumulated in the yolk, reaching a maximum equivalent to 0.20 p.p.m. of P^{32}-malathion after 7 to 9 days (average value 0.16 p.p.m.) and then slowly declining to an average of 0.03 to 0.05 p.p.m. after 20 to 30 days. In general, the residues in the eggs from the sprayed birds were about

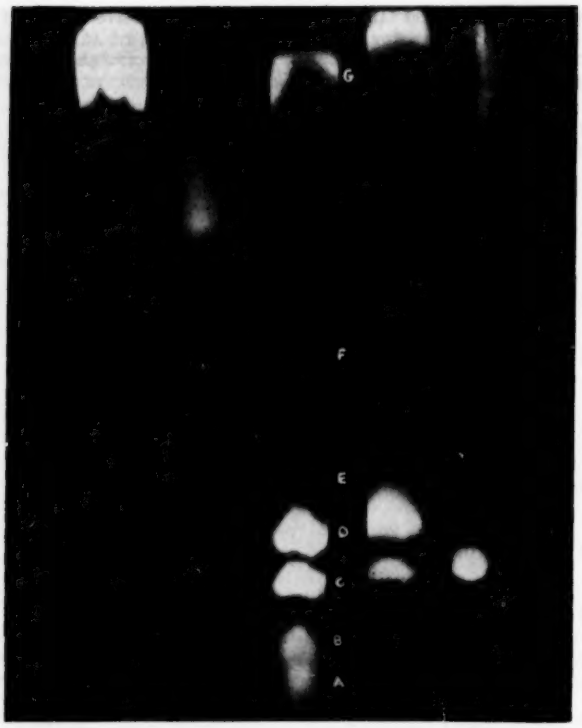


FIG. 1.—Radioautographs of chromatograms of P^{32}-malathion and metabolites. Left to right: (1) P^{32}-malathion; (2) acetone extract of feathers (Hen No. 6); (3) total water extract of 3- to 4-hour droppings from hen (see Table 9); (4) total 24-hour urine from mouse injected with 0.01 ml. P^{32}-malathion; and (5) total water extract of 2-hour gut from cockroach topically treated with 1 μl. P^{32}-malathion.

one-tenth as large as those in the eggs from the fed birds, which is about the same differential found in the two sets of tissues. It seems clear from the residues found in droppings, tissues, and eggs, as previously noted, that the actual residues of malathion and chloroform-soluble metabolites were only about 10 per cent or less of the total residues indicated.

Table 4.—P^{32}-malathion and metabolites in eggs at intervals while hens were being fed radioactive feed and after return to normal feed.[a]

Days	P^{32}-Malathion and Chloroform-Soluble Metabolites in Whole Egg, γ/g.	P^{32}-Malathion and Total Metabolites, γ/g. in Yolk	White
On radioactive feed (100 p.p.m. P^{32}-malathion)			
2	0.0054	—	—
3	0.0050	—	—
4	0.013	—	—
5	0.0096	—	—
6	0.023	0.25	0.080
7	0.055	0.92	0.17
8	0.030	—	—
10	0.049	0.57	0.12
12	0.073	0.69	0.14
13	0.10	2.14	0.22
On normal feed			
16	0.084	1.03	0.030
17	0.10	1.07	0.0030
18	0.080	1.57	0.014
19	0.074	0.93	0.0041
20	0.085	1.67	0.019
21	0.063	0.96	0
22	0.056	0.77	0.031
24	0.041	0.43	—

[a] Radioactive feed replaced by normal feed after 15 days.

Intraperitoneal Injections of Hens.—Table 9 contains data showing the rate of elimination and metabolism of malathion following the intraperitoneal injection of 0.25 ml. P^{32}-malathion. The data show that malathion is rapidly eliminated even after a massive dose, over 50 per cent of the injected dose being eliminated within 3 hours and nearly the entire dose being eliminated within 24 hours. This rapid elimination of an injected dose further substantiates the hypothesis that malathion is not very readily absorbed through the skin or from the feathers, as noted above. Only a small fraction of the original dose remained to be excreted between 24 and 48 hours. During the first hour the excreted radioactivity was almost en-

Table 5.—P^{32}-malathion and metabolites in hen droppings at intervals after spraying with 0.5 per cent emulsifiable P^{32}-malathion at the rate of 190 mg. P^{32}-malathion per hen (112 p.p.m. based on average hen weight 1690 g.).

	P^{32}-Malathion and Metabolites in Droppings, γ/g.												
	Hen Number												Average Per Hen
Days	9	3	8	1	2	4	5	6	7	10	11	12	
1	39.2[a]	19.5	17.6	13.4	23.7	27.2	30.7	16.7	43.3	15.8	19.5	31.6	24.8
2		12.9[a]	23.6	10.4	13.9	10.0	11.1	7.6	9.5	16.9	17.0	15.5	13.5
3–4			14.0[b]	9.6[c]	12.2	6.9	8.0	11.2	8.1	13.6	12.4	12.7	10.9
5–7					10.8[a]	9.1	7.3	7.9	6.7	13.6	11.2	9.0	9.4
8–9						3.3[a]	3.9	5.5	4.3	6.8	7.8	3.9	5.1
10–14							4.8[a]	6.3	2.1	3.2	3.3	4.0	4.0
15–18								2.2[a]	1.4	1.6	1.7	1.6	1.7
19–21									0.75[a]	0.81	1.4	1.2	1.0
22–24										3.1[a]	10.1	2.9	5.4
25–28											1.2[a]	0.55	0.82
29–32												2.7[a]	2.7

[a] Hen sacrificed at longest time interval shown.
[b] Hen sacrificed at 3 days.
[c] Hen sacrificed at 4 days.

Table 6.—P32-malathion and metabolites excreted per hen in droppings and average values for partition between chloroform and water of P32-malathion and metabolites in droppings of hens sprayed with 0.5 per cent emulsifiable P32-malathion at the rate of 190 mg. P32-malathion per hen.[a]

Days	Ave. Excretion of P32-Malathion and Metabolites in Droppings per Hen			Per Cent P32-Malathion and Chloroform-Soluble Metabolites in Droppings		Per Cent Water-Soluble Metabolites in Droppings	
	Mg. per Sampling Period	Mg. per Day	Accumulated Per Cent per Sampling Period	Average	Range	Average	Range
1	3.5	3.5	1.8	13	2–57	87	43–98
2	2.1	2.1	2.9	6	2–9	94	91–98
3–4	3.4	1.7	4.7	5	3–7	95	93–97
5–7	2.7	0.90	6.2	7	4–14	93	86–96
8–9	1.2	0.60	6.8	16	5–39	84	71–95
10–14	2.5	0.50	8.1	7	1–12	93	88–99
15–18	1.1	0.27	8.7	7	6–10	93	90–94
19–21	0.60	0.20	9.0	0	0	100	100
22–24	3.0	1.0	10.6	27	13–40	73	60–83
25–28	0.34	0.082	10.8	7	0–14	93	86–100
29–32	2.1	0.52	11.8	18	18	82	82

[a] 112 p.p.m. based on average hen weight of 1690 g.

Table 7.—Distribution of P32-malathion and metabolites in hen tissues at intervals after spraying with 0.5 per cent emulsifiable P32-malathion at the rate of 190 mg. P32-malathion per hen.[a]

Days	Hen No.	Fraction[b]	P32-Malathion and Metabolites in Tissues, γ/g.							
			Breast	Drumstick	Liver	Gizzard	Kidney	Blood[c]	Skin (Breast)	Feathers
1	9	T	0.10	0.22	0.54	0.092	0.53	4.8	—	—
		C	0.040	0.082	0.13	0	0.066	1.9	—	—
2	3	T	0.028	0.13	0.32	0.036	0.33	0.11	—	—
		C	0	0	0	0	0	0	—	—
3	8	T	0.18	0.062	0.51	0.084	0.92	0.13	—	—
		C	0.072	0.045	0	0	0	0.0065	—	—
4	1	T	0.015	0.054	0.22	0.034	0.31	0.043	—	—
		C	0	0.019	0	0	0	0	—	—
7	2	T	0.041	0.072	0.23	0.062	0.60	0.060	—	—
		C	0	0.059	0.063	0	0	0.038	—	—
9	4	T	0.042	0.078	0.11	0.036	0.25	0.037	—	—
		C	—	—	0.029	—	0.12	—	—	—
14	5	T	0.036	0.051	0.12	0.065	0.24	0.022	—	—
		C	—	—	0	0	0	—	—	—
18	6	T	0.077	0.048	0.13	0.077	0.21	0.047	9.0	2500
		C	0	0	0	0	0	0	—	—
21	7	T	0.051	0.051	0.041	0.061	0.11	0	15	120
		C	0	0	0	0	0	0	—	—
25	10	T	0.23	0.086	0.16	0.57	0.13	0.24	9.9	—
		C	0.10	0	0	0	0	0	—	—
28	11	T	0.10	0.33	0.17	0.057	0.20	0.029	36	—
		C	—	—	—	—	—	—	—	—
32	12	T	0.22	0	0	0	0	0	13	—
		C	—	—	—	—	—	—	—	—

[a] 112 p.p.m. based on average hen weight of 1690 g.
[b] T, maximum total residue P32-malathion and metabolites; C, maximum residue P32-malathion and chloroform-soluble metabolites.
[c] γ per ml. instead of per g.

Table 8.—P^{32}-malathion and metabolites in eggs at intervals after hens were sprayed with 0.5 per cent emulsifiable P^{32}-malathion at the rate of 190 mg. P^{32}-malathion per hen.[a]

Days	P^{32}-Malathion and Metabolites in Egg Yolk, γ/g.	Days	P^{32}-Malathion and Metabolites in Egg Yolk, γ/g.
1	0	15	0.11
2	0.020	16	0.063
3	0.040	17	0.081
4	0.11	18	0.053
6	0.12	20	0.051
7	0.16	21	0.048
8	0.14	22	0.038
9	0.15	23	0.040
10	0.13	24	0.034
11	0.13	26	0.042
12	0.12	28	0.024
13	0.11	30	0.054
14	0.094		

[a] 112 p.p.m. based on average hen weight of 1690 g.

Table 9.—Rate of elimination of P^{32}-malathion and metabolites in droppings and partition between chloroform and water after intraperitoneal injection of 0.25 ml. (ca. 300 mg.) of P^{32}-malathion into hen.

Hours After Injection	P^{32}-Malathion and Metabolites in Droppings: Partitioned Between Chloroform and Water, γ/g. Chloroform Fraction	Water Fraction	Total	Wet-Ashed with HNO_3, Total γ/g.	Total Mg. per Sampling Period
0–1	1.9	1,800	1,800	1,700	55.2
1–2	2,900	3,900	6,800	5,100	61.2
2–3	8,300	4,200	12,500	16,200	130
3–4	41	3,200	3,200	3,000	12.1
4–5	23	2,900	2,900	4,800	7.3
5–7	32	2,900	2,900	2,800	24.2
7–24	8.6	120	129	280	26.2
24–48	0.63	4.8	5.4	8.0	1.3

tirely in the form of water-soluble metabolites. This trend reversed itself during the second hour, and by the third hour the radioactivity excreted as malathion and chloroform-soluble metabolites was twice as much as that excreted as water-soluble metabolites. The trend again reversed, and after 3 hours almost all activity was excreted as water-soluble metabolites. Over 40 per cent of the injected dose was excreted between the second and third hours. This appears to indicate that unchanged malathion itself can be excreted, and that the rate of metabolism can be considerably exceeded by the rate of elimination.

The data in table 10 show the distribution of malathion and metabolites in various tissues after intraperitoneal injection. The fact that the highest residues are found in the liver and kidney is further evidence of the importance of these tissues in metabolism and excretion. In all cases residues in breast, drumstick, and brain were less than residues in the blood. This may indicate that the residues in those tissues are due to the blood which they contain rather than to storage in those tissues. The residues in liver and kidney considerably exceeded those in blood indicating a concentration of malathion and metabolites there.

Effect on the Hens.—In both the feeding and the spraying experiments no gross abnormal symptoms were noted in the hens. In the feeding experiments large numbers of dead round worms were eliminated in the droppings during the first few days on the radioactive feed. Egg laying was somewhat reduced in the feeding experiments, the hens laying at a 29% level, but this reduction may probably be accounted for by disturbance of the hens. The feeding experiment was begun the day following their transfer to the new location, and they were considerably disturbed by changing of feed dishes and removal of dropping-collection pans. In addition, the malathion-fortified feed was somewhat less acceptable to the hens than normal feed. In the spray experiment the hens laid at a 68% level, which approximates the level which might have been expected of them The higher residues of total radioactive compounds in the eggs of the fed hens than in those of the sprayed hens may, in part, be accounted for by the smaller number of eggs produced by the fed hens.

The hens intraperitoneally injected with malathion showed definite initial symptoms of poisoning, although, even at the highest level (200 mg./kg.) the hen was not

Table 10.—Distribution of P^{32}-malathion and metabolites in hen tissues at intervals after intraperitoneal injection with P^{32}-malathion.

Hours After Injection	Amount Injected per Hen: Ml.	P.P.M. Based on Weight of Hen	Fraction[a]	P^{32}-Malathion and Metabolites in Tissues, γ/g.: Breast	Drumstick	Liver	Gizzard	Kidney	Blood[b]	Brain
1.5	0.16	120	T	0.40	0.85	8.6	1.8	7.3	4.5	—
			C	0	0	0.097	0	0	0	—
5	0.17	140	T	6.0	5.1	55.7	—	93.5	17.4	3.7
			C	—	—	—	—	—	—	—
17.5	0.20	140	T	0.082	0.090	1.9	0.14	0.65	0.12	—
			C	—	0	0	0.051	0.067	0.060	—
48	0.25	200	T	0.99	0.62	6.0	—	4.8	1.2	0.87
			C	—	—	—	—	—	—	—

[a] T, maximum total residue P^{32}-malathion and metabolites; C, maximum residue P^{32}-malathion and chloroform-soluble metabolites.
[b] γ per ml. instead of per g.

prostrated or killed. For the first few hours after injection, the hens were sluggish and assumed a crouching position which is somewhat typical of sick birds. When disturbed, however, they were considerably more excitable than a normal hen. In all cases, during the first few hours the hens showed symptoms of excessive salivation. The two hens sacrificed at 17.5 and 48 hours appeared quite normal at the time of sacrifice.

Reliability of Results.—An analysis of the results obtained by the various methods used, i.e., by partition between chloroform and water, by chloroform extraction of large amounts of tissues, and by wet-ashing with nitric acid, indicate the reliability of the results. In comparing the amounts of malathion and chloroform-soluble metabolites determined on duplicate samples by the partition method and by the chloroform extraction of large amounts of tissues, residues determined by the latter method were found to be 0.75 ± 0.45 (standard deviation) times those of the former. Only those tissues from the hens given 100 p.p.m. of malathion in the feed were processed by these two techniques, and the results reported in table 3, showing the amount of malathion and chloroform-soluble metabolites, are from partition results (corrected as noted below) which were the higher of the two sets of values.

In comparing the total chloroform-soluble plus water-soluble residues obtained from the partition data, with the total residues obtained from wet-ashing with HNO_3 for duplicate samples, the residues from the latter method were found to be 1.48 ± 0.72 (standard deviation) times those from the former. Apparently, sizeable amounts of radioactive compounds remain in the tissue residuum after homogenizing and partitioning between chloroform and water. The data reported in table 3 for total residues and malathion plus chloroform-soluble residues in the tissues of the fed hens were corrected by this factor, since they were obtained by the partitioning technique. The data reported in tables 7 and 10 for total residues in the tissues of the sprayed and injected hens were obtained by the wet-ashing technique, but the malathion plus chloroform-soluble residues were obtained from partition data and were corrected as well.

All of the data on total residues in the droppings are taken from figures obtained by partitioning of the droppings between chloroform and water. They may be somewhat lower than the actual amounts present, since in a small number of cases where determinations from partitioning were compared with duplicate determinations from wet-ashing, the latter were found to be 1.27 ± 0.47 (standard deviation) times the former.

Preliminary Studies on the Metabolism of Malathion in the Hen, White Mouse and Cockroach.—The metabolism of malathion in the hen and white mouse appears to be quite complex, as shown by the number of spots on the chromatograms prepared from water extracts of the hen droppings and of the mouse urine (see Fig. 1). The pattern and number of spots are essentially the same in both.

When mouse urine obtained from the first elimination, several hours after injection with P32-malathion, was diluted with water and extracted several times with equal volumes of chloroform, the major portion of the activity remained in the aqueous phase, as shown below:

Extraction	Radioactivity (c.p.s./ml.) Water Phase	Chloroform Phase
First	73.5	16.4
Second	65.9	7.8
Third	60.8	4.4
Fourth	55.5	4.3
Fifth	53.1	3.3
Sixth	50.3	2.0

Although the activity partitioning into the chloroform phase gradually diminished it was still appreciable even after the sixth extraction. The relatively high activity in the first chloroform extract is probably due to unmetabolized malathion. The partitions between chloroform and water indicate that the metabolites are much more polar than malathion, although they can be extracted slowly out of the aqueous phase by chloroform. When to the same mouse urine was added one-fourth of its volume of 5% aqueous sodium carbonate and the whole was then extracted with an equal volume of chloroform, the activity remained in the aqueous phase in the ratio of 440:1. From this it would appear that the metabolites, or at least some of them, are acidic in nature.

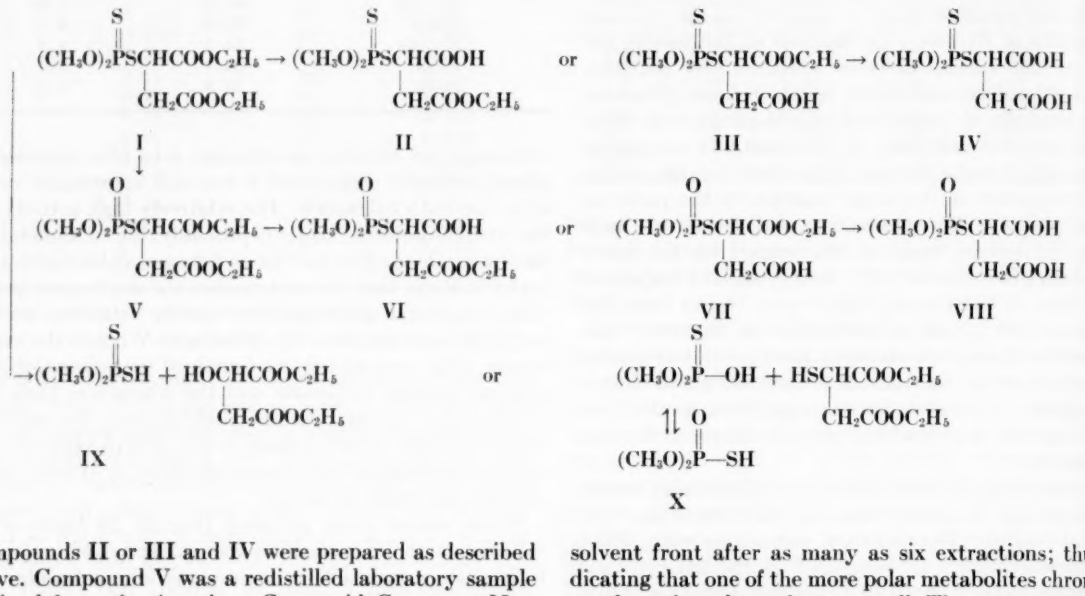
When mouse urine collected through 36 hours after injection with P32-malathion was extracted with chloroform, 458 γ radioactive compounds/ml., calculated as malathion, were found in the water phase and 10.7 γ/ml. in the chloroform phase. This shows a considerable decrease in chloroform-partitioning compounds, in comparison with those of the mouse urine collected at the time of the first elimination after treatment as noted above, and indicates more complete metabolism. When these two phases of the initial partition were re-extracted several times with chloroform or water, the following results were obtained:

Re-Extraction	Chloroform Re-Extraction of Initial Water Phase (γ Radioactive Compounds/Ml.)	Water Re-Extraction of Initial Chloroform Phase (γ Radioactive Compounds/Ml.)
First	12.3	8.5
Second	11.1	0.56
Third	11.1	0.25
Fourth	11.1	0.29
Fifth	10.9	0.32
Residual compounds	401 (Water phase)	0.82 (Chloroform phase)

These data present essentially the same information as noted above concerning the partition of the majority of the compounds present in favor of water over chloroform. In addition the re-extraction of the chloroform phase with water shows that for this sample of urine very little unchanged malathion was present. Eighty per cent of the radioactive compounds in the initial chloroform phase were re-extracted into water with a single re-extraction. The amount of unchanged malathion thus was very small, since less than 0.2 per cent of the compounds originally

present remained in the initial chloroform phase after five re-extractions with water.

From the structure of malathion it is possible to postulate possible metabolic products which can be derived by simple chemical reactions, as shown in the following schematic diagram.



Compounds II or III and IV were prepared as described above. Compound V was a redistilled laboratory sample received from the American Cyanamid Company, New York, N. Y. The structure of the half-ester (II or III) has not been proved definitely, since *0,0*-dimethyl hydrogen phosphorodithioate can add to ethyl hydrogen maleate adjacent either to the carboxyl or to the carboethoxy group.

The insecticidal and house-fly cholinesterase-inhibiting activities of compounds I, II or III, IV, and V are shown in table 11. These data indicate that the more polar compounds (II or III and IV) are less effective toxicants than malathion from which they would be derived. The thiolphosphate [*0,0*-dimethyl *S*-(1,2-dicarboethyoxy) ethyl phosphorothiolate (V)] is more toxic and might be expected to be the principal toxic metabolite derived from malathion.

The paper chromatograms in figure 1 show the separation of at least seven distinct compounds excreted in chicken droppings and mouse urine after intraperitoneal injection of P^{32}-malathion. The spot (G) at the solvent front probably contains two or more components in addition to malathion itself. Compound V, if present, would be expected to chromatograph at the solvent front along with malathion, under the experimental conditions. Chromatographs of the chloroform fractions from multiple partitions of the water extracts of hen droppings or mouse urine with chloroform show a single spot at the solvent front after as many as six extractions; thus indicating that one of the more polar metabolites chromatograph at the solvent front as well. The agreement of the number of compounds separated by paper chromatography with the number of compounds postulated as possible metabolites seems very significant. Preliminary determinations of the cholinesterase-inhibiting activity of the separated spots indicate detectable activity only in the spot at the solvent front. This agrees with the toxicity data in which the two more-polar compounds (II or III and IV) examined were less effective cholinesterase inhibitors and exhibited low toxicity. It is probable that the more-polar, water-soluble metabolites which comprise the major part of the residues found in the tissues, eggs, and droppings of the hens are relatively nontoxic.

In a series of experiments on the metabolism of Systox isomers in the white mouse and the American cockroach, no differences in the routes of metabolism were found in the two organisms (March *et al.* 1955). With P^{32}-malathion, however, the metabolic products formed differ greatly in the cockroach and mouse or chicken. The chromatogram of malathion and metabolites separated

Table 11.—Relative contact toxicity and anticholinesterase activity of malathion and some probable metabolites.

Compound	LC_{50},[a] Per Cent Weight, to Volume Solution			House Fly	
	Citrus Red Mite	Greenhouse Thrips	Mosquito Larvae	LD_{50},[b] γ/Female Fly	I_{50},[c] Molar Concn.
I (Malathion)	0.0042	0.00016	<0.00001	0.6	7×10^{-5}
II or III (Half Ester)	>1.0	>1.0	0.00035	>10	3×10^{-5}
IV (Diacid)	>1.0	>1.0	>0.01	>10	$>1\times10^{-4}$
V (Thiolphosphate)	0.0032	0.000018	—	ca. 0.6	1×10^{-7}

[a] Lethal concentration for 50% mortality in 24 hours.
[b] Lethal dose for 50% mortality in 24 hours.
[c] Concentration for 50% inhibition of house-fly head cholinesterase.

from an extract of cockroach gut (Fig. 1) contains only two spots, as compared with seven spots for extracts from mouse or chicken. This indicates a considerably less complex and less effective metabolism in the insect and may explain the great differential in toxicity for malathion between insects and warm-blooded animals. Apparently, the majority of the rapidly formed metabolites in the warm-blooded animals are of a low order of toxicity. The inability of the insect to form such metabolites undoubtedly contributes in a major way to the effectiveness of malathion as an insecticide.

Further work on the synthesis of malathion derivatives and the separation and characterization of the metabolites in warm-blooded animals and insects is in progress.

SUMMARY AND CONCLUSION.—The fate of P^{32}-labeled malathion [*O,O*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorodithioate] has been extensively studied in the laying hen and, for comparative purposes, in the white mouse and American cockroach. Hens were treated by feeding 100 p.p.m. P^{32}-malathion in the mash, by spraying with 0.5% P^{32}-malathion water emulsion, and by intraperitoneal injection. Residues were determined in the droppings, eggs, and various tissues.

In the experiments with fed hens, 60 per cent of the consumed malathion was eliminated in the droppings in 2 to 4 days and 75 per cent in 5 to 6 days. Ninety-seven to 98 per cent of the radioactive compounds excreted was in the form of water-soluble metabolites and degradation products. The maximum total residues found in any of the tissues or eggs examined were less than 3 per cent of the concentration being fed. These residues decreased after the hens were removed from the radioactive feed.

In the case of the sprayed hens, the largest concentration of radioactivity was excreted in the droppings in the first 24 hours after treatment, the amount decreasing throughout the remainder of the experiment. The maximum concentration in the droppings was only about one-half that in the droppings of the fed hens. Less than 12 per cent of the applied dose was absorbed and eliminated in 32 days; this suggests that malathion is poorly absorbed through the skin and unavailable for absorption from the feathers. About 90 per cent of the compounds excreted was in the form of water-soluble metabolites and degradation products. The residues in tissues and eggs were approximately one-tenth of those found in the fed hens.

When a massive dose was injected intraperitoneally into a hen, more than 50 per cent was eliminated in 3 hours, and nearly the entire dose in 24 hours. During the period of greatest elimination, 1 to 3 hours after the injection, nearly twice as much radioactivity was eliminated as unchanged malathion and chloroform-soluble metabolites as was eliminated as water-soluble metabolites and degradation products. At other times, 90 per cent or more was excreted as water-soluble metabolites and degradation products.

No gross toxic effects were noted in either the fed or the sprayed hens.

The metabolism of malathion in the laying hen and white mouse is complex, probably involving progressive hydrolysis of the ethyl ester moieties to produce more ionic and water-soluble compounds which apparently exhibit a low order of toxicity. It probably involves, as well, the oxidation of the thiono-sulfur moiety to produce a series of thiolphosphates. One of these [the analog of malathion *O,O*-dimethyl *S*-(1,2-dicarboethoxy) ethyl phosphorothiolate] may be the principal metabolite showing marked anticholinesterase activity and toxicity.

The metabolism of malathion in the American cockroach is apparently less extensive and complex than it is in warm-blooded animals. The less effective metabolism in the insect may explain the great differential in toxicity for malathion between insects and warm-blooded animals.

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Selective Pesticides as Aids to Biological Control of Apple Pests[1]

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Problems due to the increase of such formerly minor pests as orchard mites, the red-banded leafroller, the plum curculio, scale insects, and the yellow-necked caterpillar are only too familiar to apple growers in the eastern United States since DDT came into general use against the codling moth in 1946. As stated by Baker (1952), "Never before have so many pests with such a wide range of habits and characteristics increased to injurious levels following application of any one material as has occurred following the use of DDT in apple spray programs." These outbreaks have been attributed largely to the toxic effect of DDT on the natural enemies of certain of these pests and to the omission or reduced usage of lead arsenate and the mineral oils against others. However, very little is known concerning the importance of their natural enemies before the advent of DDT, and still other factors may have been involved in these outbreaks.

The need for long-term ecological investigation of the factors causing these disturbances became increasingly apparent, and in 1952 a project was started at Kearneysville, W. Va., to study the effects of spray practices on apple pests and their natural enemies and to develop, if possible, a closer coordination between chemical and biological control in eastern orchards (Clancy 1955). This paper describes these studies and summarizes the results obtained during 1952–1954.

Earlier studies in Virginia (Clancy & Pollard 1952) indicated that predators play an important part in keeping mites under control in unsprayed or abandoned orchards, the most efficient being predaceous mites of the genus *Typhlodromus* (formerly *Iphidulus*), but that most of the predators are destroyed by DDT and the sulfur fungicides used in commercial orchards.

Typhlodromus mites collected from apple foliage in local orchards, identified by E. W. Baker, indicate a complex consisting of *pomi* (Parrott), *fallacis* (Garman), and two slightly different forms near *fallacis* which may be undescribed species. They were also collected from numerous wild host plants in and around these orchards together with *conspicuus* (Garman) and another undescribed form. *T. cucumeris* (Oud.) was obtained only from winter collections of surface debris in the abandoned orchard.

The complexities of insect and disease control often necessitate a dozen or more annual applications of pesticides, which leave little opportunity to favor the beneficial species through reduced dosages, fewer sprays, or different timing. Furthermore, many pesticides have such long residual action that even a few applications would eliminate most of the natural enemies. Spray programs based on insecticides and fungicides that are least toxic to the beneficial forms thus seem to offer the only real prospect of increasing the biological control of apple pests. Materials that are relatively nontoxic to the beneficial forms are considered selective.

With fruit-infesting species such as the codling moth, *Carpocapsa pomonella* (L.), biological control is more difficult than with strictly leaf-feeding pests. The larvae are well protected from their natural enemies and, since only a few survivors can inflict serious economic damage, populations must be kept at extremely low levels. Biological control of the codling moth was unsatisfactory even before DDT became available (Boyce 1940, 1943; Collins 1934; Cox & Daniel 1935; Driggers & Pepper 1936; Driggers & O'Neill 1938; Jaynes & Marucci 1947; Lloyd 1944). With increased emphasis on high-quality production, insecticides will probably continue to be required for its suppression. The basic need is thus for a selective insecticide having most of the advantages of DDT without its harmful effect on the beneficial species.

The margin of control is considerably wider for species that feed on the leaves and twigs, such as mites, aphids, and scale insects, since larger populations of these pests can be tolerated without appreciable injury. This group is also attacked by many parasites and predators that generally maintain satisfactory control on unsprayed trees, but most of the natural enemies are eliminated by DDT in commercial orchards. Although the red-banded leaf roller, *Agyrotaenia velutinana* (Wlkr.), feeds on both fruit and foliage, it also is heavily parasitized and relatively scarce on unsprayed trees. Spray programs based on selective pesticides should therefore reduce the need for special applications by permitting increased biological control of these pests.

Experimental Procedure.—The initial studies at Kearneysville are conducted in a recently abandoned orchard having higher codling moth and natural-enemy populations than occur in well-sprayed orchards. Seasonal schedules are applied to 2-tree plots replicated three times in a 9-acre block of 40-year-old Black Twigs, and other trees are used for special experiments of a temporary nature. Each sprayed pair is completely surrounded by unsprayed trees which serve as guard rows and as reservoirs for both injurious and beneficial species, thus equalizing all populations and giving each treatment an unusually rigorous test. Sprays are carefully timed and thoroughly applied from the ground at an average rate of 30 gallons per tree, with conventional equipment and a single-nozzle gun. A water-sprayed plot is also included as a check on the washing effect of spray treatments. The trees have been pruned and fertilized to facilitate spray coverage and restore normal vigor. The most promising materials or combinations are then tested in larger plots of about 1 acre in several commercial orchards in comparison with standard schedules, all sprays being applied by the growers.

Mite and predator populations are sampled at biweekly or monthly intervals from May to September by collecting and brushing 90 to 120 leaves per plot and making 5-minute counts of the larger predators on each tree (Clancy & Pollard 1952). Control of the major insect pests is evaluated by means of standard fruit examinations in July and again at harvest.

Studies in 1952.—Correspondence with A. D. Pickett, of the Canada Department of Agriculture, indicated that

[1] Accepted for publication September 6, 1955. E. O. Hamstead assisted in these experiments in 1952.

in Nova Scotia lead arsenate, the fixed nicotines, and the fungicides glyodin and dichlone were less harmful than other standard materials to various natural enemies of apple pests. Consequently, 4 plots were established in the abandoned orchard in which glyodin, dichlone, captan, and flotation sulfur were included in a complete 9-spray seasonal program to determine the effect of these fungicides on predaceous mite populations. Glyodin and captan showed little or no effect on these mites, sulfur was highly toxic to them, and dichlone was moderately so. Glyodin also showed evidence of an acaricidal effect on the European red mite, *Metatetranychus ulmi* (Koch), not possessed by captan.

Glyodin was also used in 4 additional plots with the following insecticides: DDT, lead arsenate, tank-mixed nicotine bentonite, and activated ryania. Earlier ryania preparations had shown considerable promise against the codling moth (Hough & Hill 1954),[2] and it was hoped that a botanical insecticide of this type might also be less harmful to the beneficial species. As shown in table 1, only ryania proved effective against a severe codling moth infestation. No worm holes were found in the apples sprayed with it, and only 8.7 per cent bore very small stings at harvest. On the other hand, 47 to 75 per cent of the fruit sprayed with lead arsenate, nicotine bentonite, or DDT was affected. There were no marked differences in European red or predaceous mite populations in these plots during the first season the orchard was abandoned, though *Tetranychus* mites and the coccinellid predator *Stethorus punctum* (Lec.) became more numerous in the DDT plot.

Lead arsenate and nicotine bentonite also failed to control the codling moth in larger grower-cooperative plots in two commercial orchards (Table 2), indicating that these materials would have little value as selective insecticides in West Virginia.

STUDIES IN 1953.—Because of the promising results obtained with ryania in 1952, several spray schedules using either activated or pure ryania in combination with glyodin or captan were compared with standard DDT schedules in the same orchards during 1953.

Abandoned-Orchard Experiment.—As shown in table 1, both ryania products, at either 4 or 6 pounds per 100 gallons of spray, gave outstanding control of a severe codling moth infestation when used with glyodin, only

[2] Also unpublished reports of E. Gould, West Virginia Agricultural Experiment Station, and J. F. Cooper, of the former U. S. Bureau of Entomology and Plant Quarantine, Kearneysville, W. Va.

Table 1.—Results of spray experiments in an abandoned Black Twig Apple orchard, Kearneysville, W. Va., 1952-54.

Plot	Material per 100 Gallons[a]	Codling Moth Injury per 100 Apples		Fruit Affected	Red-Banded Leaf Roller Injury	Plum Curculio Injury	Mites per 90 Leaves[b]	
		Entries	Stings				Red	Predaceous
		Number	*Number*	*Per Cent*	*Per cent*	*Per cent*	*Number*	*Number*
1952								
	Glyodin plus—							
1	Lead arsenate	53	152	74.8	0.06	0.8	298	5
2	Nicotine bentonite	54	20	47.4	0.5	2.4	289	4
3	Activated ryania 6 lb.	0	10	8.7	0.5	0.8	223	4
4	DDT	107	43	72.5	0.2	20.0	191	6
5	Water-sprayed check	85	0.1	59.7	0.4	4.9	179	17
1953								
	Glyodin plus lead arsenate plus—							
	Activated ryania							
1	6 lb.	0	22	16.6	0.7	5.1	508	13
2	4 lb.	0	23	18.9	0.7	2.8	495	18
	Pure ryania							
3	6 lb.	0	19	15.6	0.8	4.9	123	86
4	4 lb.	0.1	38	26.7	0.5	4.8	155	82
	Captan plus lead arsenate plus—							
5	Activated ryania 6 lb.	0.1	43	31.5	0.8	6.8	658	22
	Pure ryania							
6	4 lb.	4	74	46.1	0.8	6.5	343	75
7	2 lb.	9	147	70.0	0.6	11.7	257	106
8	Sulfur, ferbam, lead arsenate, DDT	27	41	42.1	0.6	10.3	1300	4
9	Water-sprayed check	85	15	62.1	4.5	49.4	131	187
1954								
	Glyodin plus pure ryania 5 lb. plus—							
1	Lead arsenate	0	3	2.8	1.1	3.8	63	68
2	Lead arsenate plus nicotine sulfate	0.1	6	6.1	0.9	3.7	190	11
3	Alone	0.2	5	4.9	4.5	17.6	25	117
	Captan plus lead arsenate plus—							
4	Glyodin plus pure ryania 5 lb.	0	6	5.4	1.2	3.3	128	100
5	TDE plus DDT	8	9	15.5	1.5	5.8	1865	2
6	Water-sprayed check	18	1	16.6	6.9	31.8	17	148

[a] Ryania as indicated or DDT, 2 pounds of 50 per cent wettable powder, used in all 6 cover sprays in 1952 and 1953, and in second to sixth cover sprays in 1954. Lead arsenate used at 3 pounds in all cover sprays in 1952 and only in petal-fall and first cover sprays in 1953 and 1954. Fungicides used at standard recommended strengths. Complete 9- or 10-spray schedules used each year.

[b] Average seasonal population (eggs and active stages) from 9 or 10 biweekly counts on 90 to 120 leaves per plot, May to September.

Table 2.—Results of spray experiments in three commercial apple orchards, Jefferson County, W. Va., 1952–54.

Orchard and Variety	Year	Treatment[a]	Codling Moth Injury per 100 Apples		Fruit Affected	Red-Banded Leaf Roller Injury	Plum Curculio Injury
			Entries	Stings			
			Number	*Number*	*Per Cent*	*Per Cent*	*Per Cent*
Sandy Ridge:							
Delicious	1952	Lead arsenate	93	23	53.4	0.5	0.5
		Nicotine bentonite	148	7	70.6	2.4	10.9
		DDT	26	4	20.8	2.1	0.5
	1953	Activated ryania plus glyodin	1	8	7.2	1.2	0.5
		Pure ryania plus captan	11	27	25.9	1.5	0.9
		DDT	0.4	3	2.9	0.1	1.3
	1954	Ryania[b,c]	7	4	10.5	1.4	4.9
		DDT	4	1	4.6	0.6	5.1
Grimes	1954	Ryania[b]	0.4	2	2.2	3.3	2.5
		DDT	0	0.2	0.2	0.4	3.3
York	1954	Ryania[b]	0.3	0.6	0.8	0.8	2.6
		DDT	0	0.1	0.1	0.4	3.2
Twin Ridge:							
Black Twig	1952	Lead arsenate	345	422	99.4	0.4	0.5
		DDT	19	14	22.8	6.4	0.1
	1953	Activated ryania	0.1	10	9.2	2.4	1.2
		DDT	0.2	2	2.2	0.1	1.5
	1954	Ryania[d]	0	0.1	0.1	11.2	1.8
		DDT	0.1	0.1	0.1	0.4	1.4
Norwood:							
Golden Delicious	1954	Ryania	0.4	1	1.3	1.2	1.1
		DDT	0.2	0.1	0.2	0.1	1.9
Rome	1954	Ryania	0	0.1	0.1	3.3	0.5
		DDT	0	0.1	0.1	0.6	1.4

[a] Lead arsenate included in most petal-fall and first-cover sprays, and TDE and various acaricides in most DDT schedules.
[b] Last cover spray omitted.
[c] Pure ryania was used in all orchards in 1954.
[d] Lead arsenate omitted from first-cover spray.

one entry being found in 3741 apples from these plots. Control was less effective when the ryania was used with captan, though surprisingly few entries occurred even at the 2-pound dosage. Although ryania allowed more stings than DDT generally allows, most of them were small superficial blemishes, which would have little effect on fruit quality except in the extra-fancy grades.

The failure of DDT to control the codling moth in this orchard during both seasons was quite unexpected. Chemical analysis of the product used in 1952 showed that it contained slightly less than the stated 50 per cent of DDT, and in laboratory toxicity tests conducted by W. S. Hough, of the Virginia Agricultural Experiment Station at Winchester, it rated the lowest of 13 comparable commercial brands. The brand giving the best results in the tests was therefore used on the same trees in 1953, with considerable improvement (plot 8), although control was still far below that generally obtained with DDT. Most of the damage was caused by larvae of the second generation during August.

Though ryania was apparently of limited value against the red-banded leaf roller and the plum curculio, *Conotrachelus nenuphar* (Hbst.), the use of lead arsenate in the petal-fall and first-cover sprays prevented serious injury from these pests during 1953.

The effect of these schedules on both European red and predaceous mite populations is indicated in table 1, and the more important biweekly plot variations are shown in figures 1 and 2.

European red mite infestations were by far the highest and predaceous mite populations the lowest on the trees that received the standard sulfur-ferbam-lead arsenate-DDT schedule. In special tests in the same orchard all these materials were highly toxic to the predaceous mites, yet had very little effect on the red mites. In the water-sprayed check plot, however, there were 52 times as many predaceous and only one-tenth as many red mites during the entire season as in the plot receiving the standard schedule. The correlations between predator mortality and red mite increase in plots 8 and 9 are well illustrated in figure 1. Nearly all the few *Tetranychus* mites (*schoenei* McGreg. and *telarius* (L.)) present in the orchard were found in the plot where DDT was used.

Of the two ryania formulations tested, the pure material

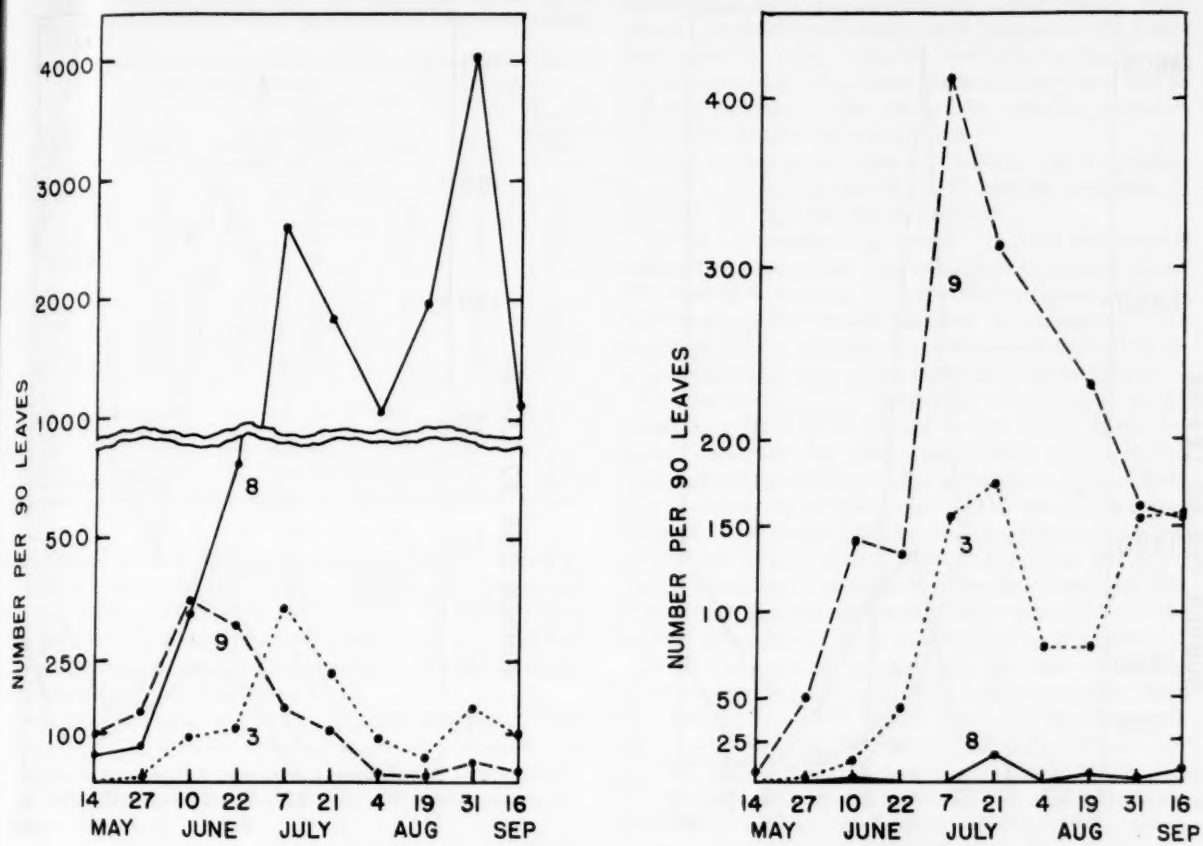

FIG. 1.—Populations of Phytophagous (left) and Predaceous (right) Mites in Plots 3, 8 and 9 of an Abandoned Apple Orchard, Kearneysville, W. Va., 1953.

apparently did not harm the predaceous mites, but repeated applications of the activated product, containing 3.2 per cent of propyl isome, seriously reduced their populations regardless of the fungicide used. In figure 2 compare the low predator levels in plots 1 and 5 with the higher levels in plots 4 and 6. There were also nearly four times as many red mites and only one-fifth as many predaceous mites in plots 1 and 2 as in plots 3 and 4. These data also confirm the 1952 results concerning the selective action of glyodin and captan, the two fungicides being about equally harmless to the predators.

Further evidence was obtained on the acaricidal effect of glyodin, especially when used at frequent intervals early in the season at the rate of 1 to 1½ quarts per 100 gallons of spray. The petal-fall and first-cover applications were the most effective ones against first-generation European red mites. The May 14 records showed an average of only 9 mites per 90 leaves in the 4 glyodin plots as compared with 100 in the check plot and 107 in the 3 captan plots, whereas on May 27 the respective averages were 11, 145, and 198. The differences decreased as the season advanced, but figures 1 and 2 show that glyodin retarded early red mite buildup in this experiment.

The importance of spray schedules based on selective pesticides to the biological control of orchard mites is indicated in the plots treated with glyodin and pure ryania, where infestations were held at about the same low levels as in the check. These favorable results were apparently due to the combined action of glyodin and the predaceous mites, fewer predators being required when the initial red mite increase was retarded by glyodin.

Besides *Typhlodromus*, other mite predators in these plots were *Stethorus punctum* (Lec.), *Leptothrips mali* (Fitch), *Hyaloides harti* Knight, *H. vitripennis* (Say), and several Chrysopidae. Only *Stethorus* was abundant enough to be of any importance. It became most numerous in those plots having the heaviest infestations, but failed to prevent the mite population from increasing, even in plot 8 where it far outnumbered the other predators. The application of DDT in this and other experiments caused only a partial temporary reduction of *Stethorus* populations.

When only DDT was applied to six previously unsprayed trees adjoining the treated ones in the abandoned orchard, the predaceous mites were practically eliminated and the European mite infestation increased nearly tenfold in 3½ weeks. On August 31, after 2½ months and three DDT sprays, there were 14 times as many red mites on these trees as on those in the water-sprayed check plot, although the few remaining leaves were in poor condition from disease infection. Essentially the same results were obtained in a similar experiment in 1952.

As reported earlier for Virginia (Clancy & Pollard 1952), these studies show that even abandoned trees of

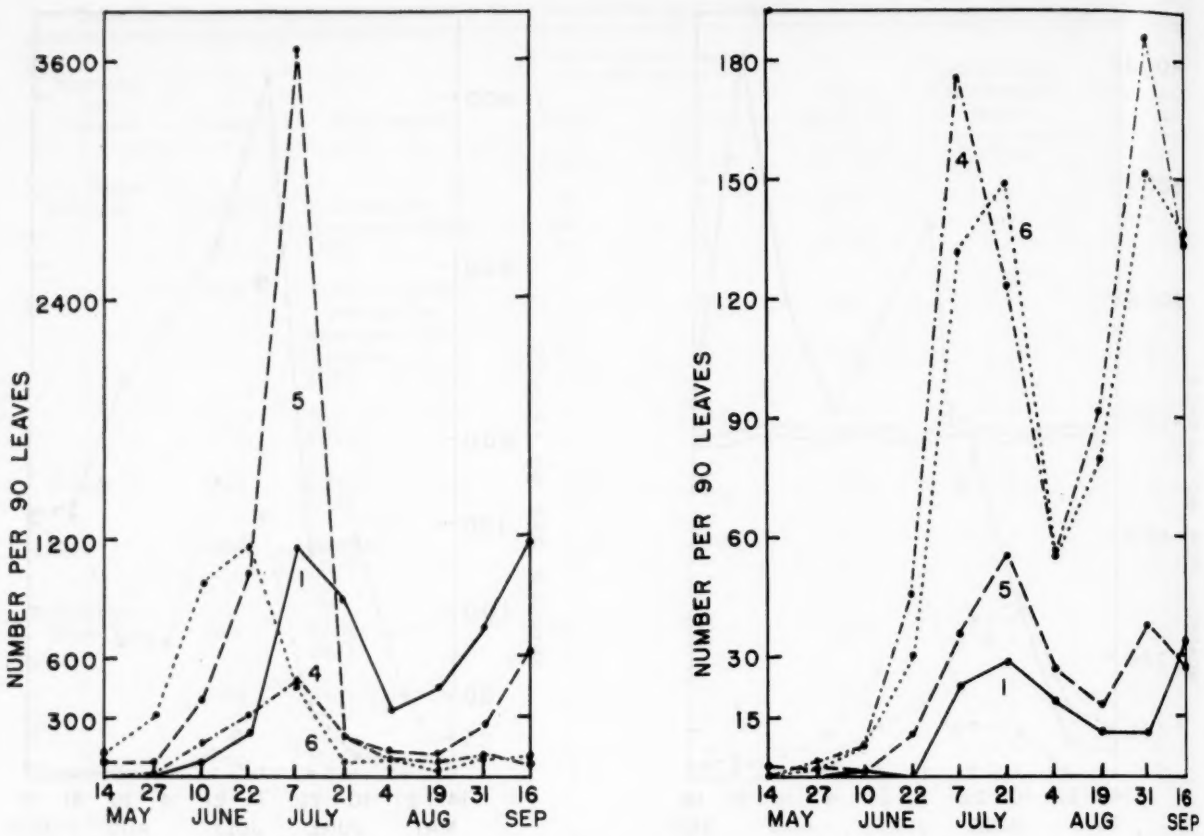

FIG. 2.—Populations of Phytophagous (left) and Predaceous (right) Mites in Plots 1, 4, 5, and 6 of an Abandoned Apple Orchard, Kearneysville, W. Va., 1953.

low vigor with poor foliage can support appreciable mite populations in the absence of effective predators.

Grower-Cooperative Experiment.—In 1953 certain of the experimental spray schedules were compared with standard DDT programs under commercial conditions, on the same trees in the two orchards used in 1952. Both ryania formulations were used at the rate of 6 pounds in 100 gallons of water, and all sprays were applied by the grower on or near the dates recommended for the area. Records were taken from 10 trees in the center of each 30- to 40-tree plot, and from 10 trees in a nearby row of each orchard that had received the regular DDT grower program.

The results of these experiments are summarized in table 2. Despite the unusually heavy codling moth infestation due to the failure of lead arsenate and nicotine bentonite in 1952, both the DDT and glyodin-activated ryania schedules gave excellent control. The reduction of larval infestation by ryania in the Twin Ridge orchard from 345 entries per 100 apples in 1952 to only 1 entry in 1953 is particularly noteworthy. Again, as in the abandoned-orchard experiment, control was somewhat less effective with the captan-pure ryania schedule.

The red-banded leaf roller and the plum curculio were controlled with lead arsenate in the petal-fall and first-cover sprays in this experiment.

Typhlodromus mites were comparatively scarce in all the commercial-orchard plots, owing mainly to the toxic effect of activated ryania and DDT, and even *Stethorus* failed to attain its former abundance late in the summer despite heavy mite infestations. An attempt to increase the *Typhlodromus* population by transferring unsprayed foliage from the abandoned orchard in mid-July resulted only in a slight temporary benefit.

STUDIES IN 1954.—Only pure ryania was used in 1954 because of its lack of toxicity to the predators. Rates of application per 100 gallons of spray were 5 pounds in the abandoned orchard and 6 pounds in the commercial orchard plots, beginning in the second cover spray.

Abandoned-Orchard Experiment.—Control of the codling moth was again outstanding with ryania but unsatisfactory with DDT (Table 1), in spite of a lighter infestation due to the unusually large crop. The consistent failure of DDT as compared with ryania in this orchard during all three seasons indicates that this population may be partially resistant to DDT. Overwintering larvae have been collected for special tests to determine whether these failures are actually due to resistance.

No benefit was obtained from the addition of nicotine sulfate to the glyodin-ryania schedule (plot 2), and the omission of the early lead arsenate sprays (plot 3) allowed increased injury from the red-banded leaf roller and the plum curculio.

Fruit infestation by the Forbes scale, *Aspidiotus forbesi* Johns., averaged only 0.5 per cent in the check and 1.1

Table 3.—Numbers of mites and eggs per 90 leaves in three plots of the abandoned apple orchard, May-September 1952–54.

Year and Month	European Red Mites			Predaceous Mites		
	DDT	Ryania	Water-Sprayed Check	DDT	Ryania	Water-Sprayed Check
1952						
May	0	5	52	0	0	0
June	63	141	376	0	0	0
July	505	495	229	13	9	28
August	24	83	26	1	3	20
September	26	70	69	8	6	29
1953						
May	65	11	122	0	2	27
June	502	107	353	1	23	137
July	1986	295	133	9	165	361
August	2295	100	24	2	104	220
September	1011	100	24	7	156	155
1954						
May	204	31	4	0	1	12
June	2263	136	43	2	18	167
July	3593	89	30	8	109	224
August	595	33	5	1	77	182
September	2668	27	2	0	135	154

to 2.2 per cent in the ryania plots, but had increased to 82.6 per cent in the DDT plot at harvest, probably owing to the destruction of its natural enemies by DDT. Other observations indicate that ryania may be effective against the apple leafhopper, *Empoasca maligna* (Walsh), and the clover mite, *Bryobia praetiosa* Koch.

In 1954 European red and predaceous mite populations showed even greater seasonal contrasts between the DDT, ryania, and check plots than in 1953. The cumulative effects of these treatments over the 3-year period are shown in table 3.

European red mites continued to increase in the DDT plot, where the predaceous mites were nearly eliminated, but decreased in the ryania and check plots (3 and 6), where the predators became about equally numerous and far outnumbered their prey. Populations reached a peak of 76 red mite stages per leaf in plot 5 as compared with only 0 9 and 0.7 in plots 3 and 6, respectively. Infestations were appreciably heavier and predaceous mites fewer when lead arsenate, alone or with nicotine sulfate, was added to the ryania schedule (plots 1 and 2), but the early lead arsenate sprays are essential against leaf rollers and curculios and the predaceous mites recovered sufficiently during the summer to prevent red mite injury. The lack of an acaricidal effect from captan as compared with glyodin also permitted a higher initial red mite infestation in plot 4. After the midsummer decline, infestations increased during September only in plots 2 and 5, where most of the predators had been eliminated by nicotine sulfate or DDT.

Stethorus punctum was again the second most numerous predator in the abandoned orchard, but it obviously had little effect on mite abundance even in plot 5, where it became most prevalent in response to heavier infestation. *Hyaloides* spp., *Leptothrips mali*, and *Chrysopa* spp. were adversely affected by DDT in this plot, but in the other plots they occurred only sporadically during the summer.

European red mite increase was usually greater following sprays of DDT than of other materials equally destructive to the *Typhlodromus* mites, suggesting the possibility of a physiological stimulus to mite fecundity by DDT, but attempts to demonstrate this effect in orchard experiments were inconclusive and further study is required. *Typhlodromus* mites were unharmed by heavy applications of DDT, demeton, and schradan to the soil, and any systemic effect these materials may have had on red mite infestation was masked by effective predation on both the treated and check trees.

Other studies on the ecology of orchard mites and their predators, including the effects of various pesticides on *Typhlodromus* spp., are still in progress.

Grower Cooperative Experiment.—In 1954 the grower-cooperative experiment was expanded to include about 170 trees of 6 varieties in 3 commercial apple orchards that received the ryania schedule in comparison with standard DDT schedules on the adjoining trees. The results obtained in these orchards are included in table 2.

Codling moth control was again about equal in the ryania and DDT plots except in the Sandy Ridge orchard, where the last ryania cover spray was omitted. Although red-banded leaf roller infestations were slightly heavier in the ryania plots where TDE was not used because of its extreme toxicity to the predaceous mites, appreciable injury resulted only in the Twin Ridge orchard, where lead arsenate was omitted from the first cover spray. Ryania apparently had some effect on the summer generation of plum curculios in this experiment.

In the abandoned orchard glyodin has repeatedly caused more injury to young fruits than has captan. However, Golden Delicious apples in the Norwood orchard and Black Twigs in the abandoned orchard were not russeted by the glyodin-ryania cover spray schedule following early captan applications, fruit finish in these plots being equal to that obtained with a straight captan schedule in the neighboring DDT plots. The ryania program thus offers considerable flexibility in the use of these fungicides prior to the second-cover spray.

In one of the commercial-orchard plots treated with ryania there were about three *Typhlodromus* mites per leaf in July, and they outnumbered European red mites during August and September, indicating successful biological control. In the other ryania plots, however, increase of these mites was apparently retarded by acaricidal sprays, though populations recovered in September and were higher than in 1953. *Typhlodromus* mites were again almost nonexistent on the DDT-sprayed trees in these orchards. Continued improvement is expected if the use of harmful pesticides can be avoided in the ryania plots.

SUMMARY.—Studies were begun in West Virginia in 1952 to study the effects of spray practices on apple pests and their natural enemies, and to seek better coordination between chemical and biological control.

Initial tests were conducted in a recently abandoned orchard where natural enemies were more numerous than in sprayed orchards. The most promising materials were also tested as complete seasonal schedules in three commercial orchards in comparison with standard spray schedules.

The fungicides glyodin and captan were found to be relatively harmless to predaceous mites of the genus *Typhlodromus*, which control the European red mite populations on unsprayed trees, but sulfur was highly toxic to them. Glyodin also showed an early-season effect on the European red mite not possessed by captan.

When DDT was used alone or in standard seasonal schedules in the abandoned orchard, predaceous mites were virtually eliminated and red mite infestations became much greater than on trees receiving any other treatment. Although DDT was very effective against the codling moth in the three commercial orchards, its failure during all three seasons in the abandoned orchard suggests that this population may be partially resistant to DDT.

A mixture of ryania and glyodin gave outstanding control of severe codling moth infestations in both the abandoned and commercial orchards, comparable to that usually obtained with DDT, but control was less satisfactory when captan was used instead of glyodin. The predaceous mites were unharmed by the pure ryania, but populations were seriously reduced by repeated applications of an activated product. The abundance of predators and comparatively low red mite infestation in the glyodin-pure ryania plots indicates the promising selective effect of this schdule in promoting the biological control of orchard mites.

Predaceous mite populations have already shown encouraging increases in several of the commercial orchard pure ryania plots, and continued improvement is expected if the use of harmful pesticides can be avoided.

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Hymenopterous Predators as Biological Control Agents[1]

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The role of predators in biological control, although less known than that of parasites, is far from negligible. The predatory activities of such families as the Carabidae, Coccinellidae, and Syrphidae have been studied and utilized in biological control, and many studies on the biology of predatory Hymenoptera have been published, but little attention has been given to their utilization in biological control.

During the last 50 years considerable progress has been made in the study of entomophagous insects, and their use in combating destructive forms has increased. The beneficial results obtained are due for the most part to a clearer understanding of the parasite-host relationship and its application to biological control. Quantities of destructive host insects, which are easily recognized, may be observed in the field or brought to the laboratory for observation. After identification and preliminary studies on the biology of the parasite and its relationship to the host, its efficiency in combating the host becomes apparent.

This method, while applicable to predators that consume their hosts *in situ*, does not apply to those that stock their nests with insects. These predators capture and paralyze their victims in the field and carry them to their nests, often some distance away. Many trips are required to stock a nest. The capture of a host by a predator is difficult to observe in the field because it often takes place at points more or less hidden from view, and the actual seizure is usually accomplished very rapidly. Even though capture of the prey may be observed, it is difficult to follow the predator to its nest unless the prey is unusually large or the nest is nearby.

The results of 20 years' observations on bees and nidifying predatory Hymenoptera in South America and France have taught me to approach the problem of locating these predators by first observing their habitats. Two factors appear to be essential in their muliplication —(1) an environment favorable to the establishment of nests, and (2) the presence within "cruising distance" of sufficient host material to stock the nest and supply the young with food.

Either of these factors is worthless without the other. However, the fertile female first locates an environment favorable for building a nest and then searches for hosts with which to stock it. Among the Pompilidae, some species construct permanent and well-built nests and then stock them with host spiders, whereas other species merely capture a spider and drag it to a makeshift cell

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or any convenient lodging place. After observing the habits of several hundred species of nesting Hymenoptera, I have concluded that if she finds no suitable environment, the female does not search for hosts.

HABITATS OF ADULT PREDATORS.—The term "habitat" is considered in this paper as the place where the adult predators establish their nests. The habitat of Anthoboscidae (=Cosilidae) and Thynnidae, which nourish their young with larvae of lamellicorn beetles, is the earthen cell of the host, which is paralyzed *in situ*. No nest is prepared in advance, but after oviposition the host cell is closed with earth or other material. More advanced predators, designated as fossorial, construct their nests in the ground, and the rubicolous forms hollow out galleries in stems of pithy plants such as blackberries, raspberries, elder, and ash. Others utilize the old tunnels of xylophagous species, and some bore tunnels in dead wood for nest construction. Still others fashion cells of mortar glued on rock, walls, or the branches of vegetation.

In general, fossorial wasps utilize three types of nest habitats—sandy soil, dead or live wood, and earth or mortar. One may encounter various predator species when examining these environments. By recognizing the cellular arrangement of the nests, the stage and number of prey in them, and the economic importance of the hosts, it is possible to establish the relation between them and the injury caused by the prey species in the vicinity. If the prey consists of destructive insects brought to the nest from a considerable distance, the devastation will not be immediately apparent.

This method of approaching any particular problem is, of course, a rather roundabout one. Nevertheless, it will eventually give general information on the habits of a considerable number of predators, which will make it possible to isolate cases of value in solving the particular problem.

Many hymenopterous predators construct their nests in sandy soil. Some species open their galleries on horizontal surfaces, and others on vertical walls in a quarry or ditch. Some species may utilize either surface.

In sandy layers of soil in France the opening tunnels of nests of *Sphex*, *Bembix*, *Mimesa*, *Oxylebus*, and certain species of *Crabro* can be found. In Chile I have seen similar nest openings of *Bembix*, *Microbembix*, and (*Monedula*) *Zyzzys chilensis* Spin. (Claude-Joseph 1928). In sand quarries the walls of vertical diggings are often peppered with small circular openings which are the nesting galleries of *Philanthus*, *Cerceris*, *Mellinus* *Xylocelia*, and *Crabro*—to cite the most common in France. *Gorytes* and *Tachysphex* nest in the walls of sandy ditches and cuts in the central valley of Chile.

Sandy soils seem to be more suitable for the nesting of fossorial species than do other types of soil. They are easily perforated and furnish at a slight depth sufficient cohesion to resist crumbling. Their permeability facilitates water drainage and allows air to circulate in the nest. These soils are also warmer than other soil types, and the cells comprising the nest become perfect little incubation chambers with absorbent walls. However, in spite of these advantages a rainy period of 8 or 10 days during the stocking period will severely damage the prey stored in the nest. They are attacked by disease and soon become a compact mass of rotting material unfit for the nourishment of the young larvae. This condition has been observed at St. Germain-en-Laye in France in nests of the following predators:

Predator	Prey
Psen ater (F.)	*Issus coleoptratus* Geof.
	Cixius nervosus (L.)
	Iassus lanio (L.)
Mimesa bicolor Shuck.	*Grypotes puncticollis* H.S.
Crossocerus quadrimaculatus (F.)	*Culex pipieus* L.
Philanthus triangulum (F.)	Bees
Cerceris rybyensis (L.)	*Halictus* sp.
Xylocelia tristis (Lind.)	Aphididae from alfalfa

Nests in a layer of sandy soil that has a layer of clay over it are in a more favorable position, as the clay prevents water from seeping into them. Some species seem to search for such protected locations. Many species exploit old walls and localities where the soil is a compact mixture of clay and sand approaching a rocky condition. *Chlorion* and *Sphex* seem to be particularly fond of rocky places. Often these insects may be observed extracting large pieces of gravel wedged into the soil, removing them to a considerable distance from the nest, and choosing others, apparently more suitable, to close the entry.

Use of Pithy-Stemmed Plants.—In South America I observed certain species of *Chilicola* and *Manuelia* (Apidae) perforating the side walls of pithy-stemmed plants. *Euplilis crassinodus* (Spin.) and *claudii* (Herbst) also make their entrance in this manner. This methed of entering the stem appears to be rare in France, as among thousands of stems examined I found only one showing such an entrance. This was a raspberry stem, and a species of *Crabronine*, after making the entry hole, constructed an ascending and descending gallery and stocked it with Diptera. Usually predators build their nests in pithy stems only after the stems have been cut.

If the stems of blackberry or other pithy plants along a hedgerow or in a forest opening are cut off with shears early in the spring, many of the stems, including the tops that have fallen to the ground, will be occupied by stocked nests of hymenopterous predators several months later. In March 1952, near the Croix de Noailles in the forest of St. Germain in France, 100 blackberry stems were cut and the tops allowed to remain on the ground where they had fallen. When these stems were examined the following July, 46 of the standing stems contained bee nests and 29 predator nests; and 22 of the fallen tops contained bee nests and 14 predator nests. Thus, in 200 possible sites 111 hymenopterous insects had constructed their nests.

In Brittany and in the Vendée (western France), where many agricultural holdings are cut up into small parcels, banks and hedgerows dividing the fields and properties are numerous. Every year the cultivators trim back by hand the plants growing in these hedgerows. These plants consist principally of *Rubus*, *Sambucus*, and *Rhamnus*. The cut portions are carried away and used for animal bedding and fuel for cooking. Most of the cut ends remaining on the hedgerows are utilized by Apidae and predaceous Hymenoptera for nest building.

Formerly vegetable gardeners in the vicinity of Paris surrounded their fields with elder (*Sambucus*), which was periodically cut back. The cutoff portions of these plants afforded bees and predators ample sites for nests and

reproduction. The solitary bees nesting in these stems visited the flowers of nearby fruit trees and aided in their pollination. The predatory Hymenoptera stored their nests with aphids, jumping plant lice, leafhoppers, and young caterpillars collected on fruit trees and truck crops. Today this practice is no longer employed. The elder bushes have for the most part been replaced by wire fences. As a result the growers complain more vehemently each year of the ravages of insects, against which they are obliged to use increasing amounts of insecticides. The elder, with its periodical pruning, afforded a perfect habitat for many insects beneficial to agriculture.

Other plants having hollow or pithy stems that are utilized by predatory Hymenoptera and bees for nesting are bogreed (*Phragmites* spp.), bamboo (*Bambusa* spp.), honey-ball (*Buddlei* spp.), willow (*Salix* spp.), snowball (*Deutzia* sp.), and wild rose (*Rosa canina*). Some of the more cellulosic plants afford a superb environment for certain predators of Psyllidae, Aphididae, and leafhoppers.

Use of Other Types.—In 1952 a severe storm in the region of Chartres-Rambouillet in France uprooted and broke the branches of many forest and fruit trees. In the dead wood of cherry, apple, and plum trees examined were found hundreds of predatory Hymenoptera galleries and cells filled for the most part with insects noxious to agriculture. The farmers in this region, known as the Beauce, grow field crops, mostly wheat, and neglect the fruit trees planted by their forebears. As a result many of the trees are malformed, with rotten branches which have not been removed. Nevertheless, they appear to be protected to a certain degree by an army of predators. If this were not so, they probably would have succumbed to their insect enemies long ago.

The mason species of predatory Hymenoptera build their nests of earth moistened with water or salivary juices. The *Sceliphron* constructs cylindrical cells, one against the other, in protected places such as under tile roofs or along walls enclosing properties or fields. On the high Andean plateau I observed a species that constructed an elaborate and well-formed nest on the metal roof of a stable.

Some species of *Odynerus* construct amorphous nests and fill the cells with lepidopterous and coleopterous insects. Their nests are often plastered against the plane surfaces of rocks and walls, in corners and on the branches of trees. A few species in the *Odynerus* complex and at least two species of the eumenid group, *Zethus dicomboda* Spin. and *Discaelius merula* Curt. (Claude-Joseph 1930), utilize bamboo, and other grassy plants with resistant stems are also utilized, the insect cleaning out the pith and separating each cell with a transverse wall of mortar.

The creation of a convenient habitat for nesting hymenopterous predators is often accidental. However, it is possible to create habitats attractive to those that are of economic importance.

In the twenties I lived for 8 years in an enclosed property of 9 acres at Tobalaba, near Santiago, Chile. This property, located at an altitude of 700 meters, was only 3 kilometers from an important range of the Andes. During my residence there I observed the insects of the region, particularly those within the enclosure. I noticed that each time the walls of the enclosure were whitewashed they attracted a considerable number of *Hypodynerus labiatus* (Hal.). The white wall was simply peppered with their nests, but a section of the same wall that had not been whitewashed had only a few nests. A bamboo hedge 60 meters long was planted in one corner of this enclosure, which also contained clumps of bushes, fruit trees, and a grapevine. After 1 year the bamboo hedge was pruned back to a height of about $1\frac{1}{2}$ meters. Several months after the pruning I found about 10 nests of *Stenodynerus scabriusculus* (Spin.) in the stems. A year later I found about 100. Each year thereafter the number of nests increased until approximately three-fourths of the stems were occupied by *Odynerus* nests.

In France I observed the same phenomenon on blackberry and elder at several places. In the St. Germain forest there are many open areas where briars grow. In 1950 I noticed in three dried, broken stems of this plant small sinuous galleries, divided into cells by lateral partitions and stuffed with young thrips. From these nests I reared several adults of the predator *Spilomena enslini* Blut. The following March I cut off 100 stems of these briars within a radius of 60 meters of the same spot, and during the summer I was able to count 41 nests of *Spilomena*. In 200 stems cut off in the same manner in the spring of 1952 I obtained 124 nests of the same species during the summer. Early in July I opened 10 of these nests and each contained from 5 to 10 cells with an average of 40 thrips per cell.

On the plateau of Rueil-Malmaison, near the forest of St. Cucufa, in early May I cut off 20 green stems of elder bordering a small field of alfalfa. Early in August I noted that all the standing cut stems and five of the fallen portions contained nests. An inventory of these stems gave 8 nests of *Ectemnius* (*Hypocrabro*) *rubicola* D. & P. stocked with Diptera, and 12 nests of *Psen* sp. and 5 nests of *Pemphredon lethifer* Shuck. stocked with aphids.

Sometimes piles of sand deposited along the sides of roads for later use remain in position long enough to attract fossorial species for nesting. Wasps are attracted to sand piles in open cultivated country where there are few suitable locations for predatory Hymenoptera to build their nests. I have seen the spider hunters of the subfamily Trypoxyloninae and several species of large Crabroninae, which prey on Diptera and aphids, build their nests in these sand piles. *Cerceris* also has been seen stocking its nests in sand piles with *Phytonomus* and *Sitona* weevils.

Along the road in the forest of Marly, I observed a newly opened sand quarry. In only one summer the vertical walls of this quarry became peppered with orifices of the nests of several predator species. These nests were found to be stored principally with Diptera.

On June 5, 1952, I observed on the island of Tudy (near Spain) several hundred *Lestica* (*Lestica*) *subterraneas* (F.) excavating galleries in a mound of sand. The galleries were not yet provisioned with insects of any kind. About 300 meters away there was a pasture containing thousands of *Cnephasia longana* Haw. At the time I could see no correlation between the two. However, on June 21 I noted at Pont Paen near Rennes, France, in another sand mound a colony of *subterraneus* containing about 50 individuals. The nests of this colony were provisioned with adults of several Microlepidoptera,

among which were *Cnephasia* sp. (undoubtedly *longana*, but owing to their condition it was impossible to identify the species), *Eucosma certicana* (Schiff.), *Tortrix loeflingiana* L., *Glyphipteryx thrasonella* (Scop.), *Crambus hortuellus* (Hbn.), and *Brachmia rufescens* (Haw.). Had I been able to examine the nests of the colony on the island of Tudy 2 weeks after my first visit, I believe I would have found these nests also filled with various Microlepidoptera, including *Cnephasia* spp., from the nearby pasture.

IMPORTANCE OF CREATING A SUITABLE ENVIRONMENT.—If an effort is to be made to create a suitable environment for hymenopterous predators, we have at our disposal a means of attracting and maintaining them. This consists of furnishing them with a convenient environment in which to build nests. Hymenopterous predators, early in their adult life, spend several days flying around the old nest, the males searching for the females, while the females look for favorable nesting sites. If the female does not find a suitable place, she dies without ovipositing.

It is easy to supply the females with environments favorable for nest building. They will also serve as nesting places for solitary bees, which are important agents in pollination. One means is to plant elder and other pithy plants along hedgerows and borders of fields and cut back the stems periodically. The placing of sand piles and the construction of ditches with nearly vertical banks would not be too difficult. Such attempts to increase the predator population in a given locality might be well worth while. Research stations might investigate the possibilities of utilizing such environments in this way to combat insect pests.

As stated before, another essential to the establishment of a predator is a sufficient supply of hosts for the young in the nests. Some species gather their prey from distant points as well as from nearby fields. Predators of the genus *Mellinus* in the St. Germain forest are capable of carrying their dipterous victim for more than a kilometer. However, the distance has some effect on the speed with which a nest can be supplied. A *Xylocelia* female whose nest was located along the Seine River near Poissy was observed to return to her nest with an aphid victim every 8 minutes. The source of the aphids was a field about 20 meters away.

Some colonies are seriously affected by the weather. As previously pointed out, continuous rains cause the insects stored in nests in sandy soil to decompose, thus depriving the immature predators of food.

Any variation either in favorable nesting sites or in the abundance of hosts, will produce a corresponding variation in the population density of the predator. If a decrease in population of a predator is due to a scarcity of hosts, and these hosts are of economic importance, their biological control has been attained. On the other hand, a decrease in predator population without a corresponding reduction in the host population may be due to a lack of favorable nesting sites or climatic conditions. If suitable environments are not available, the insects will search for nesting sites in other areas.

In areas of France where most of the favorable nesting sites are destroyed because of clean culture and the elimination of suitable native plants bordering fields, predatory Hymenoptera do not actually disappear but their populations are greatly reduced.

I am of the opinion that those predators that have become scarce in regions where the native flora has been destroyed might become more abundant and useful if they were provided with suitable nesting sites. This might apply to some insectivorous birds also, since they are usually found to be more abundant in localities where nesting sites are readily available.

Hymenopterous predators which build their nests in quarries, banks, sand piles, and similar places have various natural enemies. In Chile I have seen scorpions hiding in the nests and destroying the adults when they return to them. In France lizards, toads, and tiger beetles are the principal natural enemies of ground-nesting species.

SUMMARY AND CONCLUSIONS.—The biotic status quo of a region is the result of a struggle that has been going on for centuries and is still continuing. If stability results, even though it is considered temporary, it is due to the interplay of all the biotic factors that enter into this struggle and tends to assure the continuity of the species.

When man clears virgin land with fire and tools and prepares it for economic crops, he eliminates, at least locally, a number of the native species of flora and fauna. This abrupt change disturbs the environment and consequently the biological stability. The new crops appear to be more susceptible to attack by certain native insect pests and the damage caused by them is in direct proportion to the size of the plantings. This, of course, compels man to use insecticides and practice certain cultural methods. If a method could be worked out whereby a certain part of the original fauna and flora could be preserved, it is quite possible that the ecological stability of a region would not be completely upset and a certain degree of biological control would be maintained.

In certain agricultural regions in France, such as the French Vendée and Brittany, where cultivated crops are surrounded, sometimes too closely, by indigenous plants, there seems to be less destruction to the crops by insects than in regions, such as the Beauce, where much of the indigenous flora has been reduced to brush clumps for game protection. In Brittany and Vendée insect predators and insectivorous birds are abundant while in the Beauce they are rare.

In view of the enormous damage to our crops caused by insect pests, long-range experiments might be undertaken to determine the value of conserving a certain portion of our native flora (and fauna) in order to provide a more suitable environment for beneficial insects. If a method could be worked out whereby a certain part of the original fauna and flora could be preserved, it is quite possible that the ecological stability of a region would not be completely upset and a certain degree of biological control would be maintained.

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The Clear Lake Gnat and Its Control in Clear Lake, California During 1954[1]

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Clear Lake, California, is one of the major recreational areas of the West Coast. The economics of Lake County, of which Clear Lake is a part, is primarily based upon the recreational attraction that is created by Clear Lake. However, Clear Lake is inhabited by a small white nonbiting gnat commonly called the Clear Lake gnat (*Chaoborus astictopus* D. and S.). This bothersome gnat has, when its numbers become too great, created a tremendous adverse economic effect upon the County. Lindquist (1942, 1943, 1946, 1951), Roth (1948), and several other entomologists (Herms 1937, Deonier 1943, Walker 1949) spent several years in studying this gnat and developing a method of controlling it. As a result of this work a method of control was developed, and in September 1949 the Lake was chemically treated with 14,000 gallons of a TDE formulation. The treatment was tremendously successful and Clear Lake's large resort business once more prospered.

For almost 2 years no larvae of the Clear Lake gnat were found in Clear Lake. In July 1951, however, they were again collected by means of a plankton net. From that date on the population steadily increased until the gnat was once more affecting the county's resort business, and a second control measure was carried out in September 1954.

PRELIMINARY STUDIES.—In February of 1954 a series of six gnat larval survey courses were laid out over the surface of the lake. The purpose of these courses, which were taken at least once a month throughout the remainder of the year, was to observe possible differences in the gnat population. Prior to 1949 most of the larvae were found in the deeper waters, but the results of these 1954 surveys indicated that they were present in every part of the lake, regardless of depth. Furthermore, the population density did not vary appreciably with the depth of water. Investigations of farm ponds during the winter months showed the larvae, which live in the mud during the day, could be found at the very edges of the ponds where the water was only 1 to 2 inches deep, as well as in the greatest depths. No other major differences in the larval populations were noted.

In the spring of 1954 four light traps were installed around Clear Lake at distances from a few hundred feet up to 1½ miles from the shore. Collections were made once a week from the first of March until the end of October. Adult gnats were collected the week of May 7 and weekly thereafter until after the lake was treated in September.

The results of these collections (Figure 1) indicate that when the adult population was high the larval population was low, and vice versa. It is partially apparent from the summer increase, shown in this graph, that had Clear Lake not been treated in 1954 the gnats might have easily reached almost unbearable numbers in 1955, nearing that of 1949. The nearness to which this condition was approached even in the summer of 1954 is indicated in figure 2 showing adults clustered about a doorway light on a quiet summer evening.

PLANS FOR CONTROL PROGRAM.—On July 30 the Board of Directors for the Lake County Mosquito Abatement District decided that the Clear Lake gnat had once more reached a population level requiring a second control treatment. The latter part of September or the first part of October was the time of year when conditions were best for a successful operation. Therefore, preparations were made to undertake the 2-day control operation during this period.

It was decided to follow very closely the procedure employed in 1949 (Lindquist 1951). The Lake consists of three parts; the upper part, which is the largest (Figure 3), was divided into six sectors each a mile wide, the southeasterly part into two sectors, and the easterly part was not divided. A local pile driver was engaged to mark each sector with pilings topped with large 5 ft. × 5 ft. × 4 ft. marine plywood boxes painted with wide alternating horizontal stripes of white and international orange. The pilings were placed a mile apart, as shown in figure 3, except at the extremities of the sectors and there they were placed one-half mile from shore.

Eight barges were contracted to disperse the insecticide in 700 foot swaths within each sector.

Twenty thousand gallons of insecticide were ordered in 50 gallon drums according to the following specifications: 30 grams of TDE, technical grade (Rhothane D-3), 10 cc. of Triton X-100 emulsifying agent, and 72 cc. of xylene (crude), with a specific gravity of 1. This amount of insecticide, when pre-emulsified and introduced into Clear Lake from a pump and aspirator system gave a concentration of about 1 part of insecticide to 50 million parts of water.

By September 20 the preparations were completed and on September 24 the desired conditions existed. Therefore, the upper section of Clear Lake was treated on September 25 and the lower sections on the following day.

POSTTREATMENT OBSERVATIONS.—For several mornings following the treatment the surface of Clear Lake was covered with the dead and dying larvae, as well as partially emerged and fully emerged adult gnats that had died during the previous night. Larval surveys were made quite frequently thereafter to observe the rate and degree of kill that had been obtained.

On the third day following treatment an estimated 60 per cent of the larvae had been eliminated. On the sixth day the upper half of the lake showed nearly 91 per cent kill. On the thirteenth and twentieth days there was an approximate 99.99 per cent kill and on the fortieth day and thereafter a 100 per cent kill had been obtained so far as mud samples were concerned.

Clear Lake is also infested with a large chironomid gnat. It has not been considered a pest and the control

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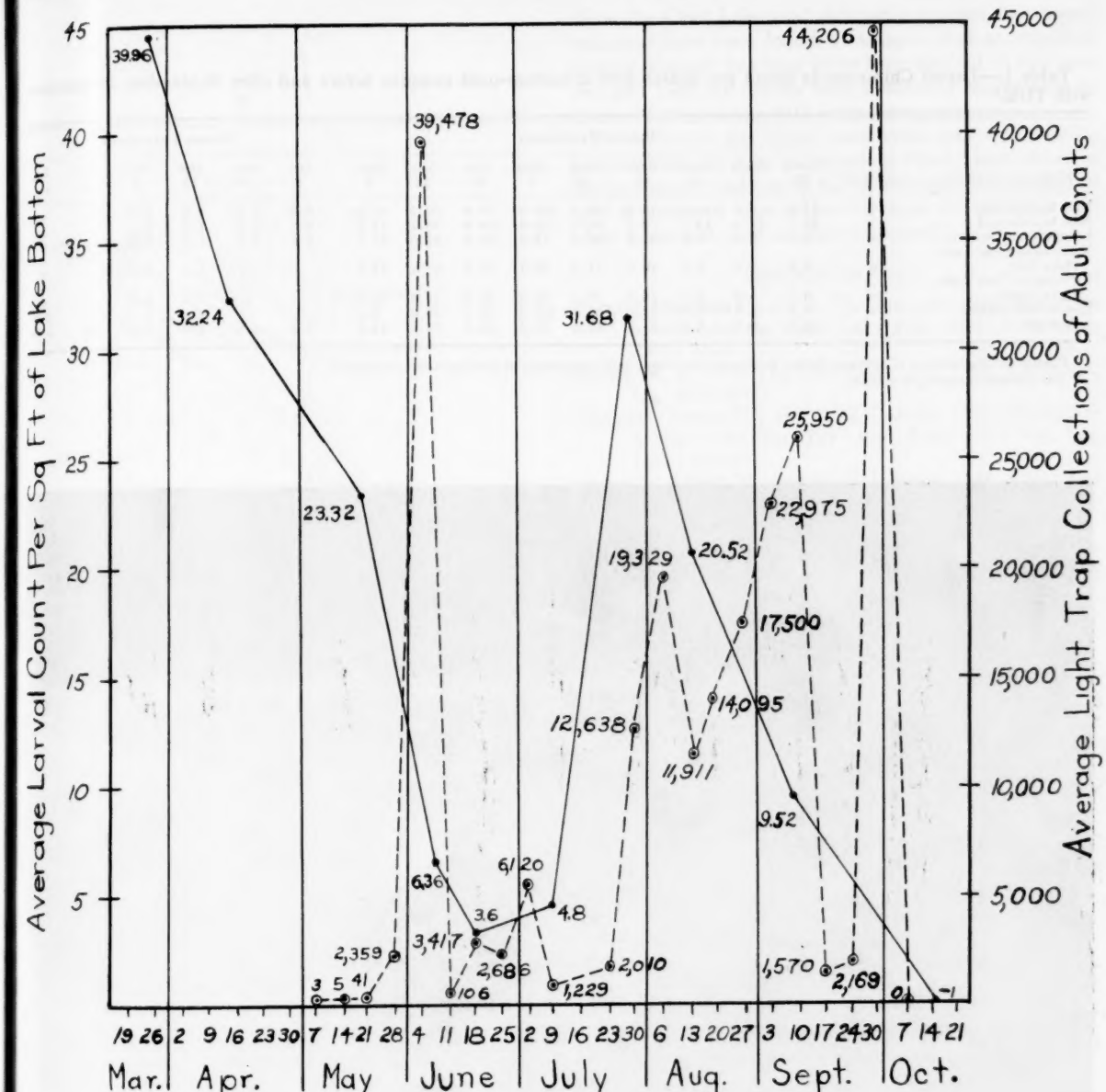

FIG. 1.—Larval (continuous line) and adult (broken line) populations of the Clear Lake gnat before and after the September 25–26, 1954 treatment.

measures were not aimed at it. It is hoped, however, that some degree of control of this gnat will be obtained since a few persons have found the mere presence of any insect to be repulsive.

The chironomid gnat population became rather high during the months preceding the 1954 treatment of Clear Lake. Fortunately the control measures exercised on the Clear Lake gnat also greatly reduced the chironomid population, although at a much slower rate (Table 1).

Observations made on the plankton and oligochaete populations indicated that these organisms had not been visibly influenced by the treatment. Apparently the vast fish population also was not harmed, for no dead fish were observed in such numbers that could not be accounted for by natural mortality.

TREATMENT OF OUTLYING REINFESTATION SOURCES.—Immediately after the treatment of Clear Lake all the sloughs, harbors, and large lakes of the area were treated. A large buffer zone was established completely surrounding Clear Lake, within which all the small farm ponds were located through the use of both ground and air vehicles. One hundred and twenty-three of these ponds or pond locations (more than one pond per location) were found and treated during the ensuing 6½ months. Probably because of the cold water temperatures during the winter months the nocturnal larval activity was reduced

Table 1.—Larval Chironomids found per square foot in bottom-mud samples before and after September 25 treatment with TDE.[a]

		Before Treatment									After Treatment				
Monthly Courses	Stations[b]	March 23	April 13	May 18	June 7	June 18	July 6	July 27	Aug. 12	Sept. 8	Oct. 1	Oct. 8	Oct. 15	Nov. 4	Dec. 15	Jan. 11
Nice to Soda Bay	7	12.9	8.0	10.3	18.6	56.0	123.2	86.0	52.4	17.2	6.0	7.9	2.3	4.6	—	0
Nice to Lakeport	7	28.0	21.4	19.7	9.7	52.8	91.0	58.6	38.4	14.6	6.6	4.3	1.4	1.1	0	0
Lakeport to Narrows	11	5.1	9.5	8.2	31.6	56.4	78.8	58.2	27.0	15.4	4.9	2.2	2.0	0.7	0	0
Narrows to Clear Lake Oaks (left)	6	6.3	7.7	4.3	10.0	13.2	48.4	24.6	27.4	16.0	—	—	4.0	2.0	—	0
Narrows to Clear Lake Oaks (right)	10	6.2	5.6	5.6	18.2	16.4	45.2	35.6	26.2	14.2	—	—	3.4	0.8	—	0
Lower Lake Section	14	5.8	4.0	5.1	58.8	58.8	108.2	68.6	26.8	8.7	—	—	2.9	1.7	—	0.3
Average		10.7	9.4	8.9	24.5	42.3	82.5	55.3	33.0	14.3	5.8	4.8	2.7	1.8	0	0.04

[a] Population fluctuations of averages before treatment conversely indicate periods of greatest adult emergence.
[b] Two Ekman samples per station.

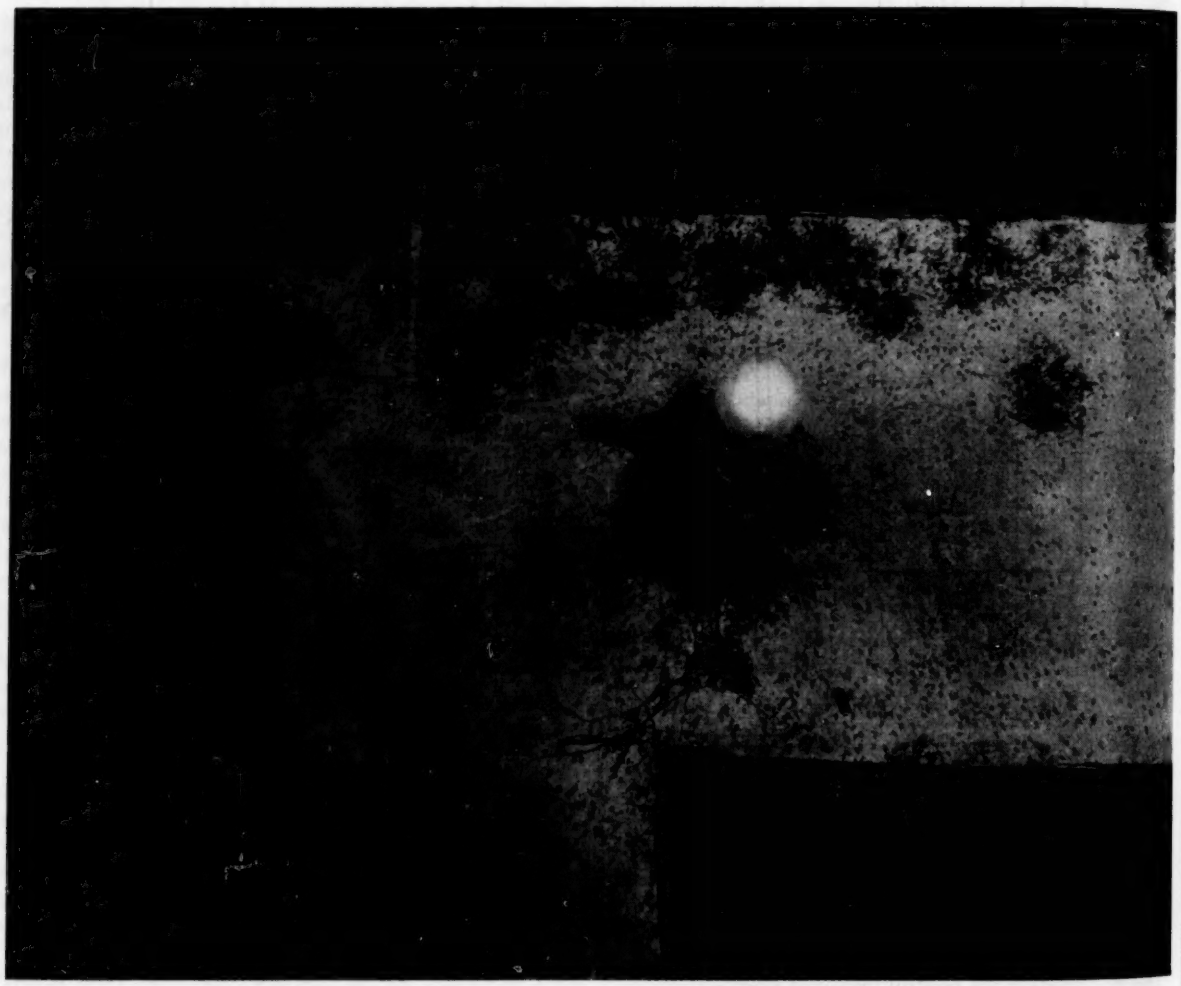

FIG. 2.—Adult gnat conditions such as this existed around Clear Lake during the summer of 1954.

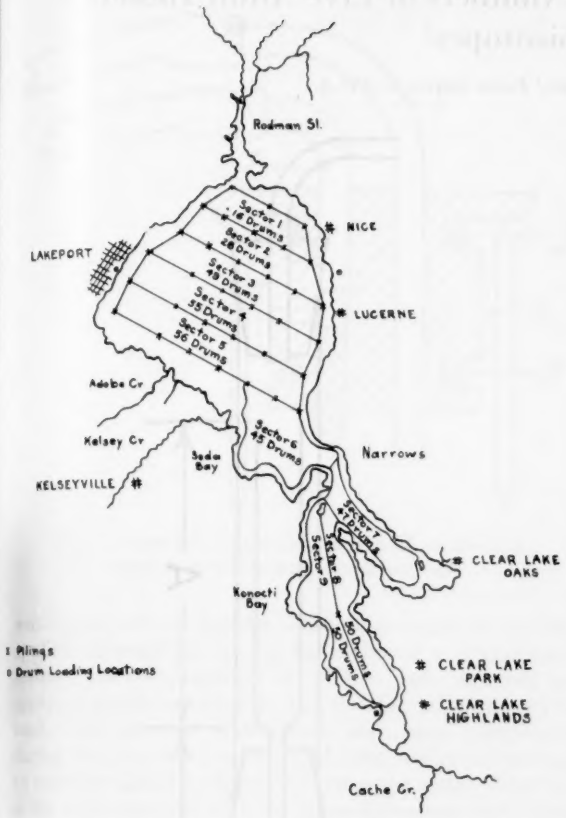

FIG. 3.—Map of Clear Lake in California showing sector boundaries and drum distribution used in treating for control of the Clear Lake gnat. Courses followed by surface craft were approximately 700 feet apart.

and one treatment did not give good control. Therefore, the ponds were treated a second time and many of them three times. When the Clear Lake gnat could no longer be found within this zone, the boundaries were extended to encompass as many more ponds as possible before the warm spring months.

CONCLUSION.—June and July 1955, the time of year when the Clear Lake gnat ordinarily appears, has passed but none have been found. It appears that an excellent control measure has once more been applied to this species and that the results were somewhat comparable to those obtained in the 1949 control program.

Even though the Clear Lake gnat may not now be found within the zone surrounding Clear Lake, either in the immature stages or in the adult stage, it is probably only a matter of time before the gnat will reappear and Clear Lake will need another treatment.

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BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Members should read this publication carefully! It sometimes contains announcements, notices, etc., not sent to the membership in any other way.

A Technique for Tagging Large Numbers of Live Adult Insects with Radioisotopes[1]

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In tracing the biology and flight habits of insects, radioisotopes have been found to be particularly useful. A small amount of either a beta or a gamma emitter used as a tag on released individuals enables the researcher to identify them upon their re-collection. Where the isotope is to serve as a means of locating hiding or burrowing insects, however, a gamma emitter is necessary because of the penetration required. In order to rapidly detect insects at convenient distances, larger amounts may be required. Techniques were devised to apply enough gamma emitter (about 20 microcuries per insect) to forest insects to permit their being rapidly located when hidden under the bark of trees or under debris on the forest floor.

In this type of experiment use of a minimum of material per insect is desirable in order that the weight of the emitter does not interfere with the insect's normal behavior. To meet this requirement, it is necessary to use material having as high a specific activity (ratio of activity to mass) as is obtainable. An added advantage in using high specific activity material is that the less the mass used per insect the smaller is the proportion of the tag that could be mechanically rubbed off or dissolved when in contact with a moist environment. For safety, selection of isotopes also was limited to those having a comparatively short half-life. In this work NaI^{131}, $Sc^{46}Cl_3$, and $Na_2Ir^{192}Cl_6$ were used successfully; whereas $Ag^{110}NO_3$ and $Rb^{86}Cl$ were toxic in the amounts necessary.

Insects may be treated individually with a tracer where only a few individuals are to be tagged; however, in experiments wherein large numbers are to be treated a mass production method is necessary. Feeding of the isotope may be the most desirable method of tagging some species, but this is not practical with insects that have long pupal stages combined with adult fasting if the isotope has a comparatively short half-life. In the work reported here, the insects were tagged by submerging them in the isotope solution.

Preliminary work indicated that with certain insects a wetting agent was necessary to get sufficient material to adhere. It was found that 20 per cent ethyl alcohol resulted in sufficient wetting and after evaporating left only the isotope and salts contained in the isotope solution on the insect. Thus, the insect's weight was not materially increased, nor were extra interfering deposits left on the insect's wings, antennae, legs, hairs, or other body parts. With several species of insects treated, including the powder-post beetle, ambrosia beetle, and the Engelmann spruce beetle, the behavior of the treated individuals could not be distinguished from the untreated under laboratory conditions.

Description of Apparatus.—Several species of insects were tagged in special glass treating tubes that were made in different sizes (Fig. 1). The dimensions of the upper part (A) of a tube were such that a batch of the insects to be treated would occupy about $\frac{1}{2}$ to $\frac{2}{3}$ of its volume (Table 1). The inside diameter of the lower part (B) was small enough to prevent the smallest individuals from getting inside it. Insects were prevented from blocking the entrance to the lower portion by dropping a piece of fine, straight wire with an "e" bent into its middle into the tube, thus obstructing the opening. Insects were then placed in the tube for treatment.

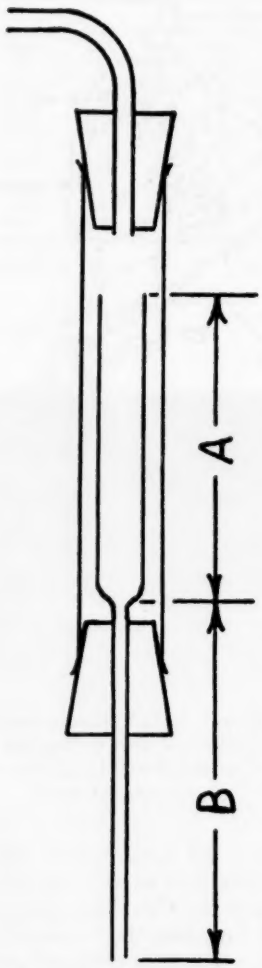

Fig. 1.—Diagram of glass treating tube in which insects were placed for application of radioisotope tracer.

The smaller treating tubes, used with small species or fewer numbers, were fitted into a glass cylinder by means of rubber stoppers, as shown. This assembly, in turn, was held in the remote pipettor. With the larger size tubes the special stopper shown in figure 2 was used, rather than the glass cylinder. With either size tubes, however, to pull the solution up over the insects contained in the upper part of the tube, the tip of the lower part (Fig. 1, B)

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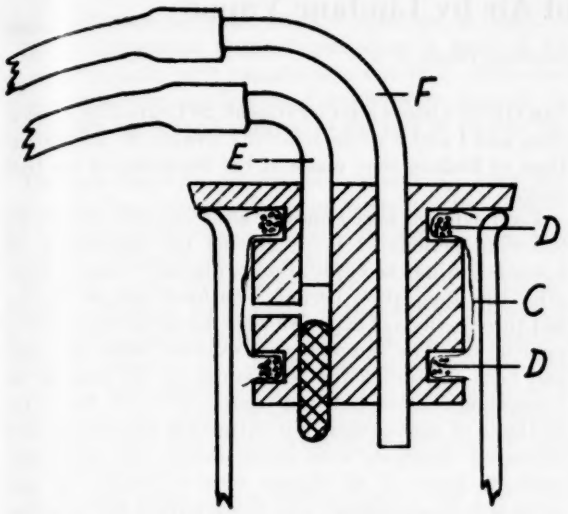

FIG. 2.—Special stopper used in large-sized treating tubes to facilitate remote manipulation.

was dipped into the isotope solution, and a gentle suction applied through the tubing by means of a hypodermic syringe. Excess quantities of solution were removed by applying gentle pressure to the syringe, which forced it back. The topmost individuals were kept submerged during this process by a weight. A lightweight vial having an outside diameter slightly less than the inside diameter of the tube was used with the larger tubes whereas a steel bearing ball having a short piece of wire soldered on for a handle was used with the smaller ones. As a matter of fact, since the density of the alcoholic solution was less than that of water and the insect density was usually greater than that of water, there was not much tendency for the insects to float.

A batch of 1000 Engelmann spruce beetles bearing 20 microcuries each represented a total of 20 millicuries. Much more activity than this is present in the treatment when the interstices are filled during treatment. To permit remote handling of this solution, the stopper illustrated in cross-section in figure 2 was designed to fit the large treatment tube. This stopper has as its main feature a remotely inflatable sleeve, which permits it to be placed and removed easily when deflated but makes a tight fit when inflated. The stopper body was turned from an ordinary two-hole rubber stopper. The sleeve (Fig. 2, C) is a section of the thumb of a rubber glove held in place by rubber bands (D). The sleeve is inflated by use of a two-valve rubber bulb connected to the tubing leading from the tube (E). When inflated, suction and pressure is applied by the hypodermic syringe through tubing leading to the tube space (F). Two tees, or preferably a tubing cross, should be inserted in the tubing near the rubber bulb. A ballast volume of about 1 pint should be placed on one branch of the cross or tee in order to steady the pressure. On the other, an inch or two of rubber tubing is placed which, during inflation of the sleeve, is pinched to close the system before pumping the rubber bulb. When deflation is desired, the pinched tubing is released.

An added, very useful refinement is the placement of a closed tube manometer in the line or within the ballast volume bottle, if of glass, to indicate the pressure. The amount of pressure required in the sleeve can then be determined in advance by trial and error. Once this is known the manometer can be used to quickly indicate the pressure in the sleeve as succeeding batches are treated.

It is advantageous when working with insects that mutilate each other while active to keep them chilled until released. With this apparatus inactivity can be achieved by placing the isotope container in an ice bath. The latter, however, should be provided with a drain so that the isotope container will not float on water accumulating from the melting ice.

This apparatus can be manipulated behind conventional shielding. If the solution is drawn to a level above the shield during the interval when the insects are submerged, one should increase the distance for protection.

EFFECT ON TREATED INSECTS.—Treated insects are dumped from the tubes onto water repellent trays to dry. These trays may be fashioned from aluminum foil with the dull side of the foil up to give the insects better footing. Trays should be placed in the shade as soon as possible after loading. As soon as the insects are dry, a few twigs should be added to the tray to serve as supports for them to climb up before taking flight. This also tends to protect the insects from birds and other predators. If possible, the trays should be placed in areas where ants and ground beetles are scarce or absent, since insects that are just recovering from a chilled alcoholic bath remain in a vulnerable stupified condition for some time.

The device described here was used in the treatment of about 19,000 Engelmann spruce beetles with I-131 in Colorado (Nagel *et al.* 1955). The beetles were treated in batches of about 1000 each, and the entire lot treated and released in about 2 hours' time. Mortality, as indicated by those remaining in the trays the following day, was about 15 per cent. This loss was not considered excessive as many were in poor health when treated. The treated beetles were fairly easily located beneath the bark of trees, by use of portable scintillation counters, for a period of about 2 weeks following the treatment. At this time the radioactivity of the isotope was only about one-fourth of its original value. About 830 (roughly 5 per cent) were later relocated by their radioactive tag. A few tagged beetles were examined by peeling the bark from over them. They appeared to be making typical galleries and laying eggs in typical numbers, indicating that little adverse effect had resulted from their treatment.

Table 1.—Approximate dimensions of tubes used in the treatment of certain insects with radioactive tracers.

INSECTS	INSECTS PER BATCH	TUBE DIMENSIONS (MM.)			
		Upper Part, A		Lower Part, B	
		Diameter	Length	Inside Diameter	Length
Engelmann spruce beetle	1,000	30	195	2.0	100
White pine weevil	100	13	95	2.0	100
Small Nitidulids	100	8	40	0.5	100

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Contamination of Food and Air by Lindane Vapor[1]

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The use of lindane fumigation devices in the home and in food storage areas has aroused speculation as to the harmful effects resulting from their use. With rapid vaporization of the lindane, considerable amounts of lindane are deposited as a residue. Foods present in the treatment area during application would be contaminated by particulate or vaporized lindane. Later lindane vapor, revaporized from the deposited residue, may be sorbed by foods present in the treated area and/or be inhaled by the occupants.

A committee on the threshold limits (American Medical Association 1954) has recommended a value of 0.5 micrograms per liter of air for 8 hours daily as the maximum concentration of lindane vapor to which workmen can be safely exposed. The saturation value of lindane vapor in air at 25° C. is 1.15 micrograms per liter; at 30° C. it is 1.8 micrograms per liter (Ethyl Corp. 1953). Such vapor saturation values are about 10 times the recommended commercial vapor concentration for continuous vaporized lindane space treatment. The actual vapor concentrations obtained in our studies rarely approached or exceeded one-tenth the saturation value for lindane. Most often they remained at or about one-hundredth of the recommended level. All vapor concentrations, except vapor concentrations during application, were below the A.M.A. recommended value.

High concentrations of lindane vapor would tend to be dissipated by air changes in the treated space. Thus a considerable portion of the vaporized lindane would be removed nearly as rapidly as it is volatilized. Theoretically, the rates of vaporization from residues deposited by different methods of application may differ due to the amount of surface per unit of lindane or the character of the residue. But, as the lindane is vaporized from the deposited residue, foods would still be contaminated regardless of the mode of application. The object of this investigation was to determine the relative contamination resulting from three methods of applying lindane.

Methods of Application.—Three conventional methods of lindane application were selected: 1, application with an electric bulb-type fumigation device; 2, an aqueous emulsion applied with a sprayer; and 3, a paint brush application of an oil solution. These methods of application are in common use for household pest control. The methods of application in this paper followed the recommendation of the manufacturer or of the United States Department of Agriculture.

Procedure.—Two series of tests were made. The rooms were selected for similarity. The walls were painted plaster, the floors varnished wood.

The food exposed during these tests was shredded wheat in packaged and unpackaged form. This material was selected because of its exceptionally high surface area. It is relatively free of solvent matter, which would tend to complicate the results with various absorption phenomena.

The food was analyzed for lindane according to the method of Schechter & Hornstein (1952). Air samples were taken following the procedure of Hornstein & Sullivan (1953) 2 hours after treatment, 26 hours after application, and 1 and 2 weeks following treatment. All applications of lindane were made at the beginning of the tests only.

Food samples were removed after exposure during the following periods: 2 hours during the application, 26 hours following the application period and 1 and 2 weeks after the application period. The food samples for the last three periods of exposure were not in the room during application. The samples were exposed after the space had been ventilated for 30 minutes in the case of the fumigation-residue but not ventilated with the other methods of application. No extraction techniques were employed. Analyses were made directly on the treated product. Rates of air change were estimated for each series of tests according to the procedures of the American Society of Heating and Ventilation Engineers (1952).

First Series.—The first series of tests was carried out as follows: (1) A total of 2⅔ grams of lindane or 8 ⅓-gram tablets were vaporized in two electric bulb-type fumigation devices in a room. Vaporization was complete in about 15 minutes but the room was kept closed for 2 hours. Following the fumigation period the room was ventilated for ½ hour according to the directions of the manufacturer.[2] The horizontal surface area of the rooms was 200 square feet. Presumably most of the lindane was deposited on the horizontal surfaces. (2) A total of 3 grams of lindane as 0.3% emulsion was sprayed on selected surfaces. These surfaces included a closet, baseboards, edges of hypothetical rugs, areas around windows and doors, corners of the rooms and under furniture. The selection of surfaces followed the recommendations of Henderson (1952). A total of 318 sq. ft. was sprayed at the rate of 9.5 mg./sq. ft. (3) Two grams of lindane were applied as a 0.5% petroleum distillate solution, with a 1-inch paint brush to selected surfaces following the directions of the manufacturer.[3] A total of 0.5 liter of the solution was applied to the baseboards, floor space under furniture, corners of the room, a closet, areas between a hypothetical carpet and the walls of the room, areas around windows and doors, cracks and crevices about the room. The total area painted was 290 sq. ft. at the rate of 6.9 mg./sq. ft.

The packages were exposed top-side up at table height. The room capacity was 1568 cu. ft. Furniture was present during the test.

Second Series.—The second series of tests was carried out as follows: In these tests only the (1) fumigation-residual and the (2) sprayed emulsion were applied. Daily temperature variations were different from those of the previous tests due to seasonal changes. The window arrangements were also different. In the first series there were 49 cu. ft. of room space per running foot of window

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[2] American Aerovap, Inc., New York City.

[3] Boyle-Midway, Inc., Chicago, Illinois.

crack, in the second series 52 cu. ft. per foot of crack.

In the fumigation residual-application a total of 1¼ grams of lindane were vaporized in one bulb. With the sprayed emulsion application 3 grams were applied. A total of 250 sq. ft. were sprayed at the rate of 12 mg./sq. ft.

The packages were exposed bottom-side up, since the bottom seal was more uniform, on aluminum foil at floor level. No furniture was in the room during the test. The volume of the room was 1070 cu. ft.

RESULTS AND DISCUSSION.—The results obtained should indicate a relationship between the decreasing lindane vapor concentrations in the space and quantities of lindane absorbed on a day to day basis. Generally, the residue on the largest surface area per gram of lindane should produce a more effective source of contamination due to the higher rate of evaporation; this residue will also be less persistent.

In the first series the application rates are 13.3, 9.5, and 6.9 mg./sq. ft. for the fumigant-residue, emulsion and oil solution residues, respectively. The second series had 11.9 and 12 mg./sq. ft. for the fumigant-residue and emulsion application, respectively.

It follows from the data in table 1 that the rates of volatilization of lindane residues are higher with the fumigation type residue than those of an emulsion and are least with a residue of lindane applied as a petroleum distillate solution. It appears, then, that the character or availability of the residue must play an important part in the relative evaporation rate of lindane from a surface.

As the air concentration of lindane vapor decreased so did the quantity of lindane adsorbed by the food (Table 2). It appears that during this period of exposure the initially adsorbed concentrations of lindane were revolatilized somewhat. Thus the concentrations obtained at the end of 1 or 2 weeks of exposure were representative of a day to day adsorption and desorption of lindane. The day to day adsorption gradually decreased with decreases in the vapor concentration. At the same time desorption from the food increases since the lower vapor concentration favors a shift in the vapor-solid equilibrium to the vapor state. With other substrates, such as products with appreciable fat content, absorption may enter into this complex.

In the first fumigation test considerable amounts of lindane were adsorbed in some of the samples. The concentration of lindane in both series was different, but package variability may have also played a role since the packaged samples in the second series, exposed with the bottom side up, showed fewer variations in adsorption of lindane vapor. The bottom flap in all packages was more uniformly sealed.

The relative rate of lindane adsorption was highest with the brushed oil application followed by the fumigation-residual, and least with the sprayed emulsion application. The parts per million of lindane adsorbed by shredded wheat on a 24-hour basis per gram of lindane applied were 3.85 p.p.m./gram lindane applied for the oil solution application, 2.56 for the fumigant-residue, and 0.6 for the emulsion application. The period during application was not included in the above results since most application directions state that foods should not be present in a space during treatment or the foods should be protected during application. These differences in contamination cannot be explained alone on the basis of quantities of insecticides applied. The surface characteristics of the residue, or the presence of quantities of a solvent, either in the food, room surfaces, or the insecticidal formulation may produce the above effects. This latter factor seems to be a cause of the large deposits of lindane found in the oil application samples. The lindane oil solution was applied in 500 cc. of petroleum distillate, which also vaporized and presumably was sorbed by the shredded wheat. The lindane, sorbed during or after this process, may have been absorbed into the oil and shredded wheat mixture. The process became typically absorptive rather than adsorptive as is presumably the case with the other two methods of lindane application. Sorption of this vapor mixture by the food would lead to an increase in concentration of lindane in the food.

The temperature was recorded during the air sampling periods—2 hours, 26 hours, 1 week, and 2 weeks after

Table 1.—Rate of decrease in lindane vapor concentrations resulting from lindane residues.

Test	Fumigant Residue		Emulsion-Residue		Oil Solution-Residue	
	µg./l.	° C.	µg./l.	° C.	µg./l.	° C.
First Series						
2 hours	3.0[a]	22.5	0.096	21	0.059	29
26 hours	0.060	21.5	0.033	21.5	0.018	28
1 week	0.015	27–28	0.008	27	0.003	27
2 weeks	0.010	30	0.006	29.5–29	0.001	20–21
Second Series						
2 hours	4.03[b]	20	0.028	20		
26 hours	0.019	25	0.006	18		
1 week	0.007	22	0.004[c]	23–28		
2 weeks	0.005[c]	20.5	0.001[c]	21.5		

[a] Three-fourths hour after starting application.
[b] One-half hour after starting application.
[c] Estimated.

Table 2.—Sorption rates of lindane vapor on shredded wheat.

	Average P.p.m. Sorbed/24 Hrs.		P.p.m. Sorbed/Percentage Saturation		Average Percentage Air Saturation	Estimated Air Changes/24 Hrs.
	Unpackaged	Packaged	Unpackaged	Packaged		
First Series						
Fumigant Residue						
2 hours	330	0	3.3	0	100	32
26 hours	2.9	18.5	0.3	1.9	9.7	43
1 week	2.3	5.1	0.4	0.9	5.6	26
2 weeks	0.72	0.1	0.2	0.04	3.4	26
Emulsion-Residue						
2 hours	3.6	4.2	0.2	0.3	16.6	32
26 hours	0.8	1.6	0.07	0.2	10.9	43
1 week	0.5	0.1	0.2	0.03	3.0	26
2 weeks	0.5	0.3	0.3	0.2	1.8	26
Oil Solution-Residue						
2 hours	7.2	2.4	2.0	0.7	3.7	32
26 hours	6.9	2.0	2.8	0.8	2.5	32
1 week	0.7	0.1	1.0	0.02	0.7	26
2 weeks	0.1	0.03	0.002	0.006	0.5	26
Second Series						
Fumigant-Residue						
2 hours	210	20	2.1	0.2	100	63
26 hours	3.6	0.3	1.8	0.2	2.0	37
1 week	0.6	0	0.4	0	1.6	22
2 weeks	1.8	0.6	1.4	0.5	1.3	15
Emulsion-Residue						
2 hours	0.5	0.5	1.0	1.0	5.6	63
26 hours	0.9	0.2	2.0	0.5	3.6	37
1 week	—	0	—	0	1.2	22
2 weeks	0	0	0	0	0.9	15

treatment. These temperatures were not recorded continuously but may be considered as indicative of the general temperatures prevailing in these rooms. Temperature changes might affect the penetration of lindane vapor into packages by alternate expansion and contraction of the air volume in the package. This factor apparently did not play a significant role in penetration in these studies, since penetration was least in the second series which had the highest daily temperature variation.

The differences in contamination in the first and second series appear to be related to air temperature variations and their effect on the vapor concentrations released from the lindane residues. In the first series the temperatures for the greatest period of food exposure averaged considerably above the temperature for the second series. From the vapor pressure (Ethyl Corp. 1953) at 30° C. there would be 1.80 micrograms of lindane per liter of space. At 20° C. there would be 0.50 micrograms per liter, a reduction of approximately two-thirds. The lindane vapor concentration differences recorded were of about that order, but the actual concentrations were much less.

Air changes per hour during comparable periods of the tests, did not vary enough to be considered of value except in the initial 2-hour exposure during fumigation. At that time the first series rate of air change was one-half that of the second series. The lower residues in the second series during this period are probably a result of this difference in changes of air per hour.

SUMMARY AND CONCLUSIONS.—Tests employing three methods of applying residual deposits of lindane were made to compare the relative contamination of food and air from lindane vapor.

The results indicate that, regardless of the method of application, food, whether packaged or unpackaged, was contaminated by lindane vapor produced from residual insecticide deposits. When similar amounts of lindane were applied employing three methods of application, the lindane vapor concentration was greatest with the fumigation-residual application, followed by the sprayed emulsion and lastly the brush applied oil solution. Food contamination was highest with the fumigation application during application; after application the oil solution deposit surpassed the fumigation and emulsion residues. Lindane applied in oil solution may lead to a more cumulative contamination than with the other two methods of application. This may be due to a solvent effect in some foods which are practically without solvent material.

On the basis of lindane vapor concentrations, as a measure of lindane residue released, the fumigation applied residue was less persistent in character than the emulsion and oil residues. Higher lindane vapor concentrations led to higher food sorption, but, as the lindane vapor concentration decreased, the sorbed contamination was reduced considerably. This reduction of sorbed lindane was probably due to revaporization of sorbed lindane and a decrease in the rate of lindane sorption.

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Marking and Trapping Studies on Dispersal and Abundance of Egyptian House Flies[1]

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From 1947 to 1951 various insecticides were evaluated under rural Egyptian conditions in a group of Giza Province villages near Cairo. In those places where chlorinated hydrocarbon insecticides were used extensively, resistant strains of house flies developed. During 1953, after a lapse of insecticide treatments, a survey showed that the flies in this area were not seriously resistant to DDT, lindane, pyrethrins, or parathion (Peffly 1955). However, flies from a previously untreated check village, Ashtar, were considerably more susceptible to lindane than were strains of flies from three nearby villages. One of these villages, Tersa, where sampled flies indicated a slight resistance to lindane, is less than 1½ miles from Ashtar. It seemed probable that migrant flies from these two villages would interbreed during the period of no insecticide treatment, with resultant increase in resistance in the Ashtar strain and a decrease in the Tersa strain.

In 1953 a study was made with marked flies and traps to obtain information on dispersal of flies from Tersa to Ashtar and other villages. In addition, marked flies were trapped in the small village of Sharawi for population estimates.

DESCRIPTION OF THE AREA.—All these villages are in an agricultural area. A heavily traveled road passes through Tersa, but Ashtar is located at the end of a side

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road 1¼ miles southeast of Tersa. There are several animal shelters but no dwellings between the two villages. Most of the inhabitants are farmers who go daily from their homes to the fields. Principal fly-breeding sources are manure piles, animal rooms, latrine dumps, fuel cakes, and human excrement, as described by Holway *et al.* (1951).

The village of Sharawi, a mile west of Tersa, is 100 meters square and has a population of approximately 300 inhabitants who keep cattle within their homes. Land owners' animals are also sheltered in an area 25 meters square adjoining the village. These situations provide ideal fly-breeding sources.

METHODS.—House flies used in the dispersal tests were collected by trap and net in Tersa over a period of 3 days. They were kept in screen-wire cages in lots of 1000 to 3000 and fed a mixture of milk and sugar. Each lot of flies was marked with a 6% solution of phenolphthalein in acetone. The solution was applied by a hand sprayer with the nozzle inside the cage. Tests showed that all flies were marked by this method.

At noon on September 28, 1953, 44,000 marked flies were released at the southeastern edge of Tersa. A sample of 400 flies taken from the cages before release contained 94 per cent of *Musca domestica vicina* Macq., 5 per cent of *Musca sorbens sorbens* Wied., and 1 per cent of *Sarcophaga* spp. Two traps were set in each of the following villages (the distances and directions are from Tersa): Madkour (½ mile southeast), Moneeb (1 mile north), Sharawi (1 mile west), Ashtar (1¼ miles southeast), Numrous (1¼ miles southwest), and Konayesa (1½ miles northwest). Screen-wire cone-type fly traps (15×9 inches) baited with fresh or partially decomposed fish were used to collect the flies. Wickets made of ¼-inch reinforcing steel were driven into the ground over the traps to prevent disturbance by animals. The trap sites were dusted with 50% chlordane to prevent ant damage to the collections. Each morning on 7 consecutive days trapped flies from each village were killed with chloroform in a covered metal cylinder. The collections were brought to the laboratory and dried in an oven at a temperature of 180° to 200° F. for 5 minutes. The catch from each village was weighed, and flies from two samples of 1 gram each were identified as to genus or species. Marked flies were detected by distributing 600 to 800 of the collected flies in a 1% sodium hydroxide solution in a stainless-steel sterilizing plate (9×12 inches). Marked flies immediately showed the purple coloring characteristic of phenolphthalein in alkaline solutions. Of damaged flies, only tagged thoraxes were counted.

During the 8-day observation period daylight temperatures ranged between 70° and 90° F. The prevailing wind of 4 miles per hour was from the northeast.

At 10 a.m. on October 21, 1953, 10,000 marked house flies, principally *M. d. vicina* and *M. s. sorbens*, were released at the center of Ezbet Sharawi to determine the relationship between the number of marked flies captured on the first day after release to the total catch. Trapping was continued for 15 additional days to obtain further information on dispersal.

To maintain population stability, flies used in the experiment were both captured and released within Sharawi. Twelve fish-baited fly traps were distributed throughout the village. They were placed on or near attractive sites such as manure heaps, latrine dumps, indoor stables, and fresh human excreta.

Table 1.—Numbers of marked and unmarked flies collected on different days after release in six villages surrounding release point.

Village	Distance from Tersa	Day	Number of Flies Collected (×1,000)	Marked Flies	
				Number	Per Cent
Madkour	½ mile southeast	1	3	9	0.3
		2	13	15	.1
		3	10	9	.09
		4	10	9	.09
		5	13	2	.01
		6	13	4	.03
		7	9	0	.00
		Total	71	48	Avg. .07
Moneeb	1 mile north	1	6	12	.2
		2	25	46	.2
		3	19	11	.06
		4	20	9	.04
		5	32	18	.06
		6	26	5	.02
		7	15	0	.00
		Total	143	101	Avg. .07
Sharawi	1 mile west	1	11	3	.03
		2	15	10	.07
		3	18	5	.03
		4	20	1	.005
		5	13	3	.02
		6	13	0	.00
		7	7	0	.00
		Total	97	22	Avg. .02
Ashtar	1¼ miles southwest	1	4	6	.1
		2	3	2	.05
		3	4	1	.02
		4	5	1	.02
		5	7	1	.02
		6	4	2	.05
		7	2	0	.00
		Total	29	13	Avg. .04
Numrous	1¼ miles southeast	1	1	0	0.0
		2	2	6	.3
		3	4	3	.07
		4	4	2	.05
		5	10	4	.04
		6	11	0	.00
		7	10	0	.00
		Total	42	15	Avg. .04
Konayesa	1½ miles northwest	1	2	1	.06
		2	2	4	.2
		3	20	4	.02
		4	10	1	.009
		5	13	0	.00
		6	16	0	.00
		7	11	1	.009
		Total	74	11	Avg. .01

RESULTS.—The results are shown in table 1. Seventy per cent of the marked flies recovered in all six villages were caught within the first 3 days. Percentages gradually decreased until the 7th day, when only one marked fly was captured. Eighty-one per cent of the recovered marked flies were collected in the three villages within 1 mile of Tersa. Schoof *et al.* (1952) recovered a similar percentage of *M. d. domestica* within a 1-mile zone.

Table 2 shows the numbers of unmarked and marked flies of *M. d. vicina*, *M. s. sorbens*, and *Sarcophaga* spp. collected on the 7 days of the experiment and table 3 the numbers collected in the six villages during the 7-day period. *M. d. vicina* comprised 78.2 per cent of all flies collected, *M. s. sorbens* 20.3 per cent, and *Sarcophaga* spp. 1.5 per cent.

A group of 12,646 trapped specimens were not considered in these figures, inasmuch as the species represented were not present in samples of marked flies examined before release. They included species of *Lucilia*, *Muscina*, and *Gymnodia*. Considerable numbers of a

Table 2.—Numbers of unmarked and marked flies caught on 7 consecutive days after release.

	Unmarked Flies			Marked Flies		
Days	*Vicina*	*Sorbens*	*Sarcophaga*	*Vicina*	*Sorbens*	*Sarcophaga*
1	19,309	6,211	478	29	2	0
2	51,340	8,338	878	83	0	0
3	62,214	11,851	1,233	32	0	1
4	53,260	16,031	943	23	0	0
5	66,565	20,513	1,011	27	1	0
6	62,088	19,817	1,092	8	3	0
7	42,043	9,842	862	1	0	0
Total	356,819	92,603	6,597	203	6	1

Table 3.—Total numbers of unmarked and marked flies of three species collected in six villages.

	Unmarked Flies			Marked Flies		
Village	*Vicina*	*Sorbens*	*Sarcophaga*	*Vicina*	*Sorbens*	*Sarcophaga*
Madkour	55,548	13,595	1,794	46	1	1
Moneeb	120,868	20,203	1,409	98	3	0
Sharawi	70,849	24,551	1,622	22	0	0
Ashtar	15,336	13,217	497	11	2	0
Numrous	30,717	10,423	743	15	0	0
Konayesa	63,501	10,614	532	11	0	0
Total	356,819	92,603	6,597	203	6	1

predatory wasp, *Vespa orientalis* L., collected in Konayesa probably reduced the number of flies collected there.

Trap catches were consistent throughout the Sharawi experiment with the exception of those on October 25 and 27 (Table 4). The low catches on these days may be attributed to extremely high winds. The 362 marked flies collected on the day after release were 50.6 per cent of the total number of marked flies collected during the 16-day experiment.

Discussion.—It appeared that dispersal of flies was not entirely by flight. Although Moneeb and Sharawi are equidistant from Tersa, about five times as many marked flies were recovered in the former village. This probably was due to the fact that the road from Tersa to Cairo passes through Moneeb. Flies, being naturally attracted to beasts of burden, human beings, and farm produce, could easily have alighted on passers-by in Tersa and rested on them as they went to or through Moneeb. Another reason for the lower catch in Sharawi may be that, by road, the distance to Sharawi from Tersa is about twice that to Moneeb.

Of the 44,000 marked flies released in Tersa, only 210, or about 0.5 per cent, were recovered. This does not compare very favorably with the 4.6 per cent of radioactive flies that Lindquist *et al.* (1951) recovered in their experiment in Oregon. However, house flies comprised only 67 per cent of Lindquist's released population, whereas 94 per cent of those released in this test were of this species. Our recovery of marked flies was about the same as that of Schoof *et al.* (1952), who on different occasions released 31,000 and 56,000 house flies tagged with radioactive phosphorus at Phoenix, Ariz., and recovered 227 and 284 of them in 50 traps.

The similarity of the present study to the studies of Lindquist and Schoof and their co-workers suggests that the Egyptian house fly disperses at about the same rate as does *M. domestica* in America, and predominantly within a 1-mile radius of the release point.

The day-to-day ratio of *sorbens* to *vicina*, as shown in table 2, was between 1 to 3 and 1 to 6. However, this does not give an accurate picture of the relative abundance of these two species in all six villages (Table 3). It is of interest to note that four villages in which no insecticides had been previously used for fly control had higher percentages of *sorbens* than the two in which insecticides had been extensively used. This suggests that *sorbens* may develop less resistance to chlorinated insecticides than *vicina*.

This study has shown that resistant flies from Tersa do become dispersed in the surrounding villages. The numbers going to Ashtar, however, may not be great enough to exert any appreciable influence on the resistance of the Ashtar strain to insecticides. Studies in population genetics and dynamics are necessary before this situation can be evaluated.

In Sharawi, on the basis of the relationship between recaptured marked flies and the total catch on the first day (362 to 28,354), it was estimated that the total house fly population on that day was between $\frac{3}{4}$ and 1 million. A total of 715 marked flies, representing 7.15 per cent of the released population, were recovered during the 16 days of the experiment. Collections contained marked flies every day until the 12th day, and one marked *sorbens* was recovered on the 16th day. The fact that about 50 per cent of the marked flies recovered were collected after the first day suggests that more than this percentage of the released population must have remained in the

Table 4.—Number of flies caught in Sharawi on different days after release, October 21, 1953.

	Total Number (×1,000)				Number of Marked Flies Recovered			
Date	*Vicina*	*Sorbens*	Other	Total	*Vicina*	*Sorbens*	Others	Total
Oct. 22	20.5	6.8	1.0	28.3	278	77	7	362
23	19.2	8.8	1.2	29.2	135	78	8	221
24	15.4	4.1	0.9	20.4	40	17	4	61
25	2.5	0.4	.2	3.1	6	2	0	8
26	20.7	5.7	.8	27.2	7	4	1	12
27	3.4	1.1	.2	4.7	2	0	1	3
28	20.0	6.7	.8	27.5	24	10	2	36
30	14.6	3.2	.8	18.6	3	1	2	6
Nov. 2	18.9	4.0	1.2	24.1	3	2	0	5
4	24.1	1.9	.9	26.9	0	0	0	0
6	12.2	2.4	.6	15.2	0	1	0	1
Total	171.5	45.1	8.6	225.2	498	192	23	715
Per Cent	76	20	4	—	69.6	26.8	3.6	—

area. Sharawi is so located that traffic would not be a major factor in fly dispersal, but the abundance of attractive materials may have been a deterrent to migration.

General observations of flies congregated on children's faces, animals, latrine dumps, and stables indicate that reduction of the fly population was rather small. Daily removal of 22,000 flies by 12 traps from an estimated population of $\frac{3}{4}$ to 1 million is not considered satisfactory for the control of flies.

A sample of 500 flies taken from the cages of marked Sharawi flies before release contained 50 per cent of *vicina*, 38 per cent of *sorbens*, 1.5 per cent of *Muscina stabulans*, 9.4 per cent of *Sarcophaga* spp., and 1.1 per cent of *Lucilia* spp., whereas of the 225,000 flies collected during the test, 76 per cent were *vicina*, 20 per cent *sorbens*, 1.25 per cent *stabulans*, 1.8 per cent *Sarcophaga*, and 0.95 per cent *Lucilia*. This difference may have been due to an insufficient number of insects for the determination of species in the pre-release sample. On the other hand, the percentage of each species of recovered marked flies corresponded closely to the percentage in the total catch.

SUMMARY.—A study was made with traps and marked flies to obtain information on the dispersal of resistant house flies from one village near Cairo, Egypt, to others in the imediate area. Phenolphthalein was used to mark 44,000 flies, 94 per cent of which were *Musca domestica vicina* Macq., 5 per cent *M. sorbens sorbens* Wied., and 1 per cent *Sarcophaga* spp. Two traps were set in each of six villages within $1\frac{1}{2}$ miles of Tersa. Marked flies were detected by immersing collected flies in 1 per cent sodium hydroxide solution.

Of the marked flies recovered in all six villages 70 per cent were caught within the first 3 days, and 81 per cent were collected in three villages within 1 mile of Tersa. Dispersal of marked flies was apparently not entirely by flight. Resistant flies did migrate from the point of release to other villages in the surrounding area but probably not in sufficient numbers to affect the resistance of the strains in these villages.

In another test 10,000 marked house flies, principally *M. d. vicina* and *M. s. sorbens*, were released in Ezbet Sharawi, and it was estimated from the first day's catch that the total house fly population in Sharawi was between $\frac{3}{4}$ to 1 million. In all, 7.15 per cent of the marked flies were recovered during the experiment. Daily collections were positive for marked flies until the 12th day, and one marked fly was caught on the 16th day.

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Outbreak of a Leaf Miner on Apple and Its Control[1]

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An outbreak of the unspotted tentiform leaf miner (*Callisto geminatella* Pack.) in apple orchards of northern Virginia occurred in 1954 and again in 1955. Chemical control measures (Hough & Hill 1955) employed against the pest during the season of 1955 contributed to a reduction of the foliage damage as compared to 1954.

Prior to 1954 the unspotted tentiform leaf miner had not been known as an important apple pest in Virginia, but a closely related species, the spotted tentiform leaf miner (*Lithocolletis crataegella* Clem.), occurred in outbreak proportions during 1944 and 1945. This species was more or less common through several seasons thereafter when its life history was studied and control measures were developed by Beckham *et al.* (1950). Haseman (1916) published on the life history of the unspotted tentiform leaf miner as observed in Missouri. The small, oblong egg is deposited on the lower surface of the leaf. The larva enters a leaf immediately under the point of attachment of the egg and as far as is known feeds only on the tissue between the leaf surfaces. The larva, when full fed, emerges from its mine and makes a cocoon which is usually on the upper surface along the leaf edge or in a fold at the leaf tip, but it may be found on the lower surface of the leaf in folds along the leaf edge. The slate-colored moth is not a strong flier. To a limited extent it is attracted to molasses-bait solution such as is used in trapping leaf-roller moths and codling moths. Winter is passed in the pupal stage in cocoons found on fallen leaves. In 1955 nearly all of the spring-brood moths emerged between the time the apple buds first showed green and full bloom.

Although the outbreak of *Callisto geminatella* in 1954 was largely confined to orchards in northern Virginia, in 1955 the insect appeared in several other states (West Virginia, Maryland and Delaware) in sufficient numbers to necessitate inquiry about its control. Severity of the outbreak in Virginia stimulated evaluation of some insecticides that were developed since nicotine sulphate

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Table 1.—Experiments to control larvae of *Callisto geminatella* in the mines of apple leaves, showing results as determined 4 days and 3 days after spraying in 1954 and 1955, respectively.

Treatment and Amount per 100 Gallons	Date Sprayed	Larvae in 50 Leaves		Control Per Cent
		Total	Alive	
15% parathion 1.5 lb.	9- 2-54	60	0	100
40% nicotine sulphate 0.75 pt.	9- 2-54	67	13	80
25% malathion 1.5 lb.	9- 2-54	71	23	67
BHC (10% gamma) 2 lb.	9- 2-54	72	56	21
25% TDE concentrate 1 qt.	9- 2-54	70	56	18
50% dieldrin 1 lb.	9 -2-54	58	47	17
50% DDT 2 lb.	9 -2-54	66	61	7
Unsprayed		68	67	
15% parathion 0.75 lb.	7-18-54	108	6	94
Systox 1 pt.	7-18-55	82	10	87
25% malathion 2 lb.	7-18-55	101	48	52
40% nicotine sulphate 1 pt.	7-18-55	102	65	35
50% DDT 2 lb. & 25% TDE 1 qt.[a]	7-13-55	143	126	11
25% Diazinon 3 lb.	7-18-55	120	110	7
Ryanicide-100 6 lb.[b]	7-18-55	136	132	2
Unsprayed		128	127	
Am. Cyanamid 3911 1 pt.	7-22-55	101	12	87
FW-293 1 lb.[c]	7-22-55	120	112	3
Unsprayed		136	131	

[a] Applied by the grower.
[b] 100% Ryania powder, S. B. Penick Co.
[c] 25% wettable powder, active ingredient 1, 1-bis (chlorophenyl) trichloroethanol from Rohm and Haas Co.

became established as the treatment to control this and other leaf miners attacking apple foliage. Preliminary evidence obtained in 1954 indicated that effectiveness of some insecticides was related to the stage of development in which the insect occurred at the time treatment was made. In other words, a knowledge of the seasonal history of the insect was essential to development of efficient control treatments. Studies of the life history initiated in 1955 are incomplete, but apparently at least four broods of larvae may develop in the apple foliage in the latitude of Winchester. Certain information on control measures obtained during the past two seasons is presented with the thought that the data may be of interest to others.

MOTH-KILLING SPRAYS.—Experiments on moth-killing properties of some insecticides were carried out in a severely infested orchard in 1954. The tests were made during August when large numbers of moths were present. Sheets were placed under the trees from which moths were collected within 1 hour of the time of spraying. Only moths showing signs of life were taken from the sheets. A sample of 100 moths from each treatment was placed in battery jars for subsequent observation. The following treatments at the indicated dosage per 100 gallons destroyed all of the moths within 24 hours: 15% parathion 1.5 lb., 25% malathion 2 lb., BHC (10% gamma isomer) 2 lb., 50% dieldrin 8 oz., 25% TDE concentrate 1 qt., 50% DDT 2 lb., 40% nicotine sulphate 0.75 pt. Although hundreds of moths were knocked down on the sheets by all the previously mentioned sprays, we were able to collect only 26 moths from sheets placed under the tree sprayed with water alone and only seven of these died within 24 hours. Temperature at the time of applying the first six insecticides mentioned ranged from 84° to 86° F. and was 74° F. for nicotine sulphate and water alone.

TESTS AGAINST LARVAE.—Comparative effectiveness of some insecticidal sprays against larvae in the mines is summarized in table 1. The results are based on microscopic examination of the contents of the mines in 50 leaves selected from each plot. Similar examinations made several days later than those reported in table 1 did not alter evaluation of any of the treatments. At the time the tests were made in 1954 a majority of the larvae were in the early instars although some in the last instar were present. In 1955 most of the larvae were in the late instars during the time of the experiments. It will be noted that parathion was highly effective both seasons and that nicotine sulphate appeared to be more effective in 1954 than in 1955. Malathion was not very effective either season and the other treatments were even less effective, except Systox and American Cyanamid 3911 (0,0-diethyl S-isopropylmercaptomethyl dithiophosphate) both of which gave promising results in 1955.

TESTS AGAINST COCOONS.—Experimental sprays were applied on the foliage early in August when many individuals were in their cocoons. Leaves having one or more cocoons were selected from each treatment and placed in battery jars for subsequent observations on moth emergence. Fifty leaves were taken from each sprayed plot and 83 leaves from the checks. After moth emergence was completed, each cocoon was opened and examined under a microscope. Approximately half of the individuals which died in the cocoons were in the pupal stage; a few had not transformed and the remainder were parasites. The proportion of cocoons containing dead parasites was not available for parathion, but was 19, 26 and 21 per cent for malathion, Systox and the unsprayed cocoons, respectively. Evaluation of effectiveness of the sprayed treatments was based on prevention of moth emergence and the results are summarized in table 2. It will be noted that parathion was the most effective insecticide used against the cocoons.

DISCUSSION AND SUMMARY.—Insecticides used effectively to destroy moths of the unspotted tentiform leaf miner included parathion, malathion, BHC, dieldrin, TDE, DDT and nicotine sulphate. Experience has shown that insecticides, such as DDT or TDE, effective against only the leaf-miner moths do not control the pest when their use is limited to cover sprays as recommended in Virginia. None of the insecticides tested as moth-killing agents, except parathion, consistently exhibited high toxicity to the larvae in mines of apple foliage. Next to parathion in order of effectiveness in 1954, when a majority of larvae were in the early instars, was nicotine sulphate, but in 1955 when most of the larvae were in the later instars at the time of spraying only 35% control resulted. Two acaracides (Systox and Am. Cyanamid

Table 2.—Effect of some insecticidal sprays applied against cocoons of *Callisto geminatella* on apple foliage, showing results as determined by emergence of moth subsequent to treatment on August 2, 1955.

Treatment and Amount per 100 Gallons	Cocoons Sprayed	Moths Emerged		Control Per Cent
		No.	Per Cent	
15% parathion 2 lb.	97	2	2	92
Systox 1 pt.	144	10	6	76
25% malathion 2.5 lb.	142	20	14	51
Unsprayed	241	70	29	

3911) exhibited promising results against the larvae in tests of 1955. When applied against cocoons on the foliage, parathion was 92% effective in preventing emergence of moths while Systox and malathion were only 75% and 51% effective, respectively. Greater latitude in effective use of an insecticidal spray against this insect is possible with parathion than with other insecticides mentioned, because parathion is highly effective against moths, larvae in the mines and individuals in cocoons at the time of spraying. Growers' experience with parathion against the leaf miner confirms this conclusion. In fact, evidence is available indicating that a single application when foliage injury becomes evident may be adequate.

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The Bionomics of Certain Insects Associated with Oak Wilt[1] with Particular Reference to the Nitidulidae

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In 1952, when investigations were begun in West Virginia on the relation of insects to the spread of oak wilt (*Endoconidiophora fagacearum* Bretz) the list of potential vectors was large. It soon became evident, however, that those to be considered seriously could be restricted to a comparatively few orders. As Leach (1940) has pointed out an insect, in order to be an effective vector, must be consistently associated with diseased plants in a way that is conducive to contamination with the pathogen and it must also visit healthy plants under conditions suitable for transmission of the disease. Early in 1952a, Leach *et al.* reported the constant association of nitidulids with the sporulating mats of *E. fagacearum* and recognized that these sap-feeding bettles also normally inhabit fresh wounds on healthy trees where infection is likely to occur. They predicted that these insects would be found to be vectors of oak wilt and this hypothesis was proved correct in 1953 by Dorsey *et al.* in West Virginia and by Norris (1953) in Iowa. These results have since been confirmed by Himelick *et al.* (1954), McMullen *et al.* (1955), and Thompson *et al.* (1955).

The nitidulids not only serve as vectors of oak wilt, but as demonstrated by Leach *et al.* (1952b) and True *et al.* (1952), they also serve as agents of spermatization by transmitting endoconidia from mats of one sexual compatibility type to mats of opposite type stimulating the production of perithecia and ascospores. *Endoconidiophora fagacearum* is a heterothallic, hermaphroditic, self-sterile fungus (Hepting *et al.* 1952). It produces two kinds of spores, endoconidia and ascospores. The endoconidia are produced asexually and have two distinct functions. They may serve as ordinary spores in dissemination and disease production, but they also function as male gametes in sexual reproduction. The ascospores are formed in shortnecked, flask-shaped perithecia as the result of a sexual process. There are two so-called compatibility types of the fungus that are morphologically alike. These have been designated type A and type B, respectively. These two types are each bisexual, but self sterile. That is, a mycelium of either compatibility type produces both endoconidia that serve as "sperm" and also perithecial fundaments with female receptive hyphae. The endoconidia produced by the mycelium of one compatibility type will not fertilize the receptive hypae of its own mycelium or those mycelia of the same compatibility type, but will fertilize those of the opposite type. Thus before perithecia and ascospores can be formed on a mycelial mat of the A compatibility type it must be spermatized by endoconidia from a mycelium of the B type and *vice versa*. In nature, since the two types are rarely intermingled (Barnett & Jewell 1954), ascospore production usually depends upon the transportation of the endoconidia from one mycelial mat to another by insects. This relationship is graphically illustrated in figure 1.

Although other insects have been made to transmit the disease under experimental conditions, (Griswold 1953, Griswold & Bart 1954), the authors consider the nitidulids to be important vectors in nature. Since the nitidulids associated with oak wilt have not previously been recognized as having much economic importance, they have not been studied extensively. If we are to gain a complete picture of the relation of the nitidulids to the spread and development of oak wilt it is desirable that we know more about their life habits. This paper presents the results of a study conducted from 1952 through 1954 on the bionomics of several species of insects commonly associated with oak wilt with particular reference to the Nitidulidae.

In the absence of any information about insect transmission of oak wilt most insects found on an oak tree killed by wilt were, in the beginning, subject to suspicion. A preliminary survey showed that the most numerous insects found on or in diseased trees were Coleoptera, but occasional representatives of Hymenoptera, Diptera, Corrodentia, Hemiptera, Lepidoptera as well as mites (Acarina) were present.

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[2] The authors appreciate the assistance of Drs. V. G. Lilly and H. L. Barnett with certain mycological and physiological aspects of these studies. F. F. Jewell, a graduate assistant, through his researches regarding insect transmission of the oak wilt pathogen and his maintenance of insect bait trapping tests gave invaluable help. Several specialists of the Entomology Research Branch, U. S. Department of Agriculture, assisted in the identification of insect specimens.

In a search for insect vectors of oak wilt it was reasonable to assume that insects consistently associated with the fruiting structures of the pathogen were more likely to be vectors than those not so associated. Nevertheless the well known role of scolytid beetles in transmitting the Dutch elm disease and the sap stains of conifers made it necessary to consider them. Therefore in the early work on insect transmission in West Virginia, attention was given to this group. *Monarthrum fasciatum* (Say) was the most abundant species associated with trees killed by oak wilt in West Virginia. Only a few specimens of *M. mali* (Fitch) and *Pseudopityophthorus pruinosus* (Eichh.) were present. It was thought that insects reared from wilt-infected trees might be contaminated with the oak wilt pathogen although extensive attempts to isolate it from the scolytids gave only negative results. The fact that scolytids, in feeding and breeding, bore into the sapwood of oak trees would make them effective vectors provided they were consistently contaminated with the pathogen. For these reasons a series of inoculations was attempted using scolytids that had been reared from trees killed by oak wilt. In some experiments the insects were caged in a manner similar to that reported by Dorsey *et al.* (1958) over blaze-type wounds and in others they were caged on uninjured bark of healthy red oak trees. In still others they were caged over holes in the bark made with steri-

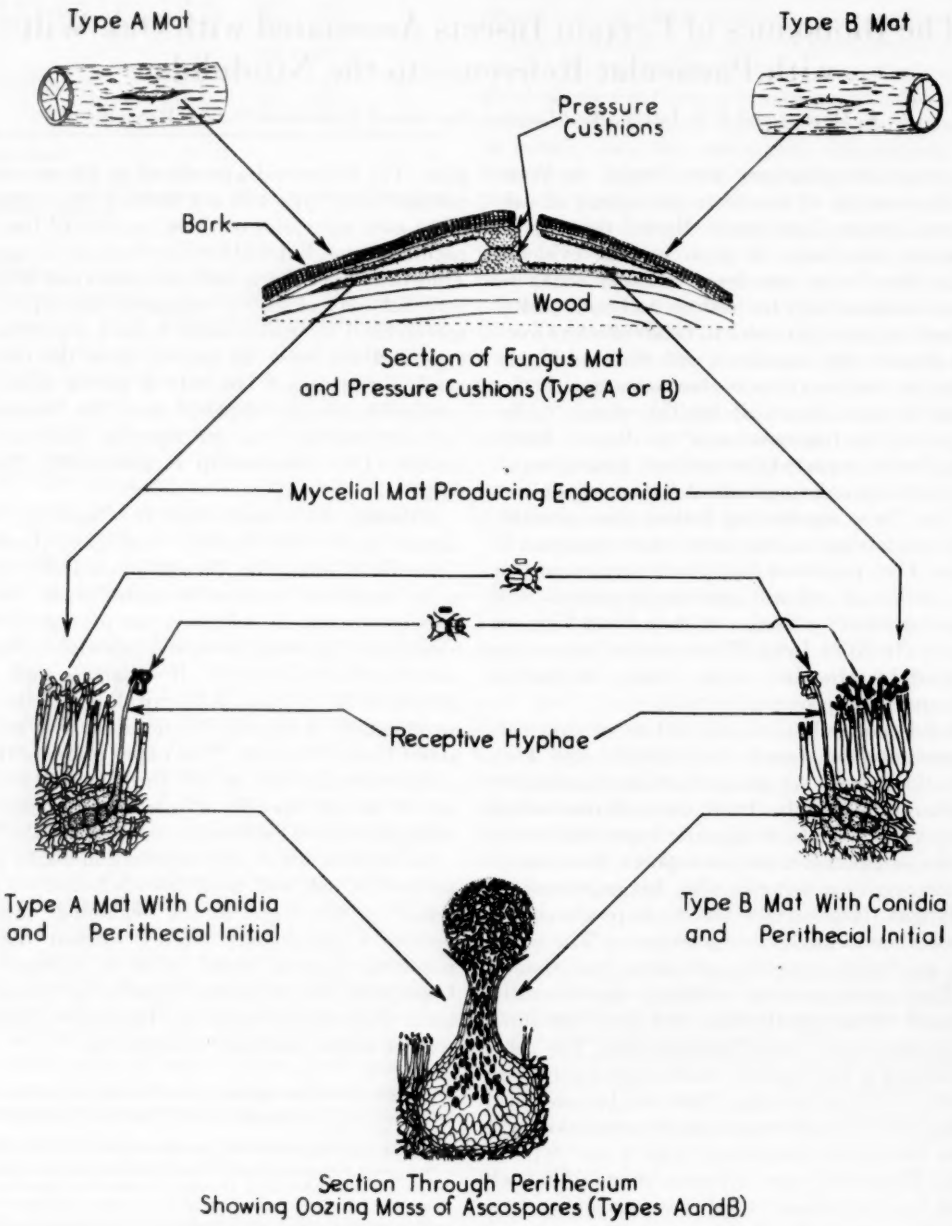
Type A Mat
Type B Mat
Pressure Cushions
Bark
Wood
Section of Fungus Mat and Pressure Cushions (Type A or B)
Mycelial Mat Producing Endoconidia
Receptive Hyphae
Type A Mat With Conidia and Perithecial Initial
Type B Mat With Conidia and Perithecial Initial
Section Through Perithecium Showing Oozing Mass of Ascospores (Types AandB)

FIG. 1.—A diagrammatic representation of the role of nitidulids in the spermatization of *Endoconidiophora fagacearum*.

lized nails to simulate scolytid entrance holes. In all, four separate inoculations involving 60 specimens of *P. pruinosus* and 30 inoculations involving 300 specimens of *M. fasciatum* were made, with negative results. In no case did the scolytids penetrate the bark of the healthy oak trees. Apparently *M. fasciatum* attacks only weakened or dying trees. This characteristic greatly limits its effectiveness as a vector of oak wilt even if it were contaminated with the pathogen when it emerged from a tree killed by oak wilt. For these reasons it was concluded that *M. fasciatum* is probably of little importance as a vector of oak wilt and special attention was given in subsequent studies to insects more closely associated with the mycelial mats. Further studies should be made however with the true bark beetles found associated with the disease. This is especially true of *Pseudopityophthorus pruinosus* which is known to attack small branches and to feed on leaf axils of healthy trees (Griswold & Neiswander 1953).

A typical mycelial mat under the bark of an oak wilt tree usually will be inhabited by a half dozen or more families of Coleoptera, a few representatives of other insect orders and other arthropods. Figure 2 shows a typical colony of insects on a mycelial mat. On one such mat representatives of nine coleopterous families (Nitidulidae, Colydiidae, Orthoperidae, Scolytidae, Histeridae, Staphylinidae, Rhizophagidae, Serropalpidae and Mycetophagidae), one dipterous family (Drosophilidae) and one family of Corrodentia (Psocidae) and mites were collected.

During 1952 and 1953 many oak wilt trees were inspected at intervals and collections were made from each tree. Table 1 summarizes the kinds of insects collected, specific collection sites on the tree and the condition of the tree. Curl (1955) lists the species of insects associated with mycelial mats of oak wilt that he observed during a 10-month period. Most of these were Coleoptera and the most numerous were representatives of the family Nitidulidae.

Late in May 1952 and again in 1953 single large red oaks (24" d.b.h.) dying from oak wilt were felled each year, sectioned (trunk and large limbs) and placed in a cubical (8′×8′×8′) insect-tight, light-proof, ventilated plywood rearing cage. This cage was equipped with glass fruit jar emergence ports on four sides. Insect collections were made from these jars twice weekly during the summer in 1952 and once a week in 1953. Table 2 presents the results of these rearing and collection studies of insects associated with individual oak wilt-infected trees.

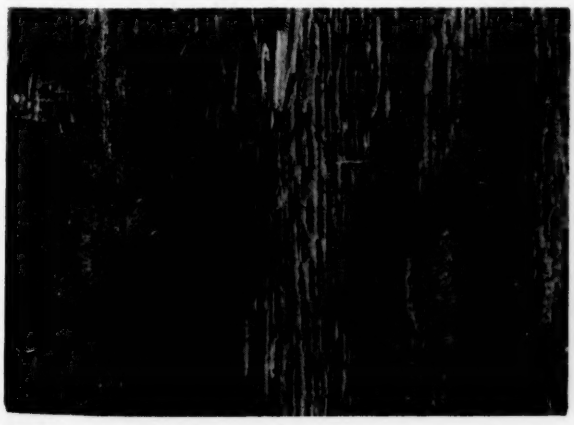

FIG. 2.—Exposed oak wilt mycelial mat on tree trunk and on inner surface of bark. Several species of nitidulids are present.

INSECTS OBSERVED ON WOUNDS IN HEALTHY TREES IN THE FOREST.—Many woody plants are injured each year by wind, lightning, ice, feeding activities of insects, and activities of man and other animals. In the spring and early summer many of these wounds become infested with nitidulids and other insects that are attracted by tree sap and fungi and with certain predaceous species. These infestations are sometimes established within a few hours in a favorable season. The fresh wounds in living trees soon become contaminated with fungi, bacteria and yeasts and apparently become for a short time increasingly attractive to certain insects, particularly the nitidulids.

For the purpose of studying the comparative attractiveness of various types of wounds to insects during different seasons, a series of trees was wounded. The reactions of the various insects that were attracted to this type of environment were observed. During the first part of May, 1953 a series of trees (oaks and maples) was wounded by ax blazing which penetrated to the sapwood. Within 4 days after the wounds were made they were heavily populated by insects, mainly nitidulids. These wounds remained attractive to nitidulids for almost 3 weeks or until most of the exposed sapwood under the loosened bark became dry. After the adult Nitidulidae became scarce in the wounds their larvae were often abundant and continued to feed freely upon fungi and other micro-organisms present in certain areas of the wound.

The study of the attractiveness of wounds in healthy trees to insects was continued in 1954 when two types of wounds were used; one was the blaze type (Figure 3) and the other was a bruise type. Both types of wounds were made with an ax. The wound studies were started in February and were continued through July. Approximately eight trees were wounded by each method each month.

No appreciable number of insects could be found in the wounds until May 13 at which time nitidulids began to appear in numbers (Jewell 1955). By June 1 these wounds contained very few adult insects. Norris (1953) found that the natural spread of the oak wilt pathogen to wounded oaks in Iowa was highest in trees wounded between May 11 and 22. The exposed surfaces of blaze wounds were dry, but the edges were still moist and they were inhabited by nitidulid larvae. By the end of June most of the larvae and all of the adult nitidulids had disappeared from the wounds.

Trees wounded during the first part of June attracted very few insects; wounds made during July attracted none. During May and early June, the period of greatest attractiveness of the tree wounds, the blaze and bruise type wounds were equally attractive to the beetles. In the first week of June 1954 a severe windstorm damaged many trees. On July 15 a number of these trees were inspected and some nitidulids were found in bark wounds. It was evident that the older wounds attracted fewer insects than the fresher wounds. However, since the

Table 1.—Principal adult Coleoptera associated with oak wilt-infected trees 1952 and 1953 in West Virginia.

Adult Insect	Collection Site	Condition of Oak Host
NITIDULIDAE		
Glischrochilus quadrisignatus (Say)	Under bark on tree	Dead
G. fasciatus (Ol.)	In bark cracks over oak wilt fungus mats	Dead
G. sanguinolentus (Ol.)	Same	Dead
G. confluentus (Say)	Same	Dead
Colopterus semitectus (Say)	Under bark on trunk and large branches on mycelial mats[a] with perithecia	Dying
C. morio (Er.)	Same	Dying trees and in freshly cut oak logs
Carpophilus lugubris Murr.	Under bark on trunk on mycelial mats	Dying
Carpophilus sp.	Same	Dying
Epuraea spp.	Same	Dying
Prometopia sexmaculata (Say)	On bark of trunk	Dead
Cryptarcha ample Er.	Same	Dead
SCOLYTIDAE		
Pseudopityophthorus pruinosus (Eichh.)	In wood of trunk and branches	Dying and dead
Monarthrum fasciatum (Say)	In bark and wood of trunks and branches and on mycelial mats	Same
M. mali (Fitch)	Same	Same
Xyloterinus politus (Say)	Under bark on trunk on mycelial mat	Dead
COLYDIIDAE		
Ten unidentified genera	On trunk and under bark	Recently killed
Cerylon sp.		
Bitoma sp.	Under bark on trunk and large branches on mycelial mats	Dying and dead
Brontis sp.	Same	Dying and dead
STAPHYLINIDAE		
Olophrum obtectum Er.	Under bark on trunk	Dying
Philonthus (4 species)	Under bark on trunk on mycelial mats	Dead and dying
Siagonium punctatum (Lec.)	On bark of trunk	Dying
Conurus sp.	Same and on mycelial mats	Same
ALEOCHARINAE		
Three unidentified genera	Same	Same
ORTHOPERIDAE		
Laemophloeus (4 species)	Under bark on trunk	Dead and dying
Brontes sp.	Under bark on trunk on mycelial mats with perithecia	
RHIZOPHAGIDAE		
Rhizophagus (4 species)	Under bark on trunk and large branches	Dead and dying
Schizophagus sp.	On trunk	Dying
PHYTHIDAE		
Rhinosimus sp.	Under bark on trunk and large branches	Dead and dying
TENEBRIONIDAE		
Helops aerus Germ.	On bark and trunk	Dead
Alobates pennsylvanicus (De G.)	Same	Same
Xylopinus saperdioides (Ol.)	Under bark on trunk on mycelial mat	Dead
One species of *Platydema*	Same	Dead
OSTOMIDAE		
Three unidentified genera	On bark of trunk	Dead
Tenebroides sp.	On mycelial mats	Dying
CURCULIONIDAE		
Cryptorhynchus pumilis Boh.	On bark of trunk	Dead
Acamptus rigidus Lec.	Same	Same
Cosson concinnus Boh.	Under bark on trunk on mycelial mats	Dead
CARABIDAE		
One unidentified genus	On bark of trunk	Dead
HISTERIDAE		
Platystoma sp.	On bark of trunk and mycelial mats	Dead
Paromalus sp.	Same	Dead
Acritus sp.	On bark of trunk and mycelial mats	Dying
Isomalus sp.	On bark of trunk	Same
CUCUJIDAE		
Silvanus sp.	On bark of trunk	Dead
BRENTIDAE		
Arrhenodes minutus Drury	Same	Same
CERAMBYCIDAE		
Clytinni—unidentified genus	On trunk	Dying
DERODONTIDAE		
Unidentified genus	On trunk	Dying
CLERIDAE		
Phyllobaenus unifasciata (Say)	Same	Same
BUPRESTIDAE		
Agrilus sp.	Same	Same
CRYPTOPHAGIDAE		
Cryptophagus sp.	Same	Same
One unidentified genus	Same	Same
CHRYSOMELIDAE		
Epitrix cucumeris (Harris)	Same	Same
Epitrix sp.	Same	Same
MORDELLIDAE		
Mordellistena sp.	Same	Same
Anaspis sp.	Same	Same
PSELAPHIDAE		
One unidentified genus	Same	Same
DASCILLIDAE		
One unidentified genus	Same	Same
SERROPALPIDAE		
One unidentified genus	On bark of trunk	Dying
Serropalpus sp.	Under bark on mycelial mat	Dead
PLATYPODIDAE		
Platypus compositus (Say)	Under bark on trunk	Dead
Platypus sp.	Same	Same
MYCETOPHAGIDAE		
Mycetophagus (3 species)	Under bark on trunk on mycelial mat	Same

[a] All references to fungi and mycelial mats pertain to *Endoconidiophora fagacearum* Bretz.

wounds were made in an excessively dry season the flow of sap continued for a short time only and the wounds tended to dry with age. Morris *et al.* (1955) found no correlation between age and attractiveness of wounds; they pointed out that the amount of sap exuded was the most important factor and this was associated with weather conditions favorable to a high degree of physiological activity at the time of wounding.

Fungus growth was commonly observed both in experimental and natural tree wounds. Specimens were removed, cultured, and the fungi proved to be mainly *Graphium rigidum* (Pers.) Sacc. and *Ophiostoma pluriannulata* (Hedg.) (Jewell 1955). Adult and larval nitidulids feed voraciously on these fungi both in natural habitats and in the laboratory.

The nitidulids were the most numerous of all insects found in the wounds of living and dying trees and of these *Carpophilus lugubris* Murr. and *Colopterus semitectus* Say were the predominating species. Both of these species have been shown to be capable of transmitting oak-wilt to healthy oak trees (Dorsey *et al.* 1953).

The Coleoptera collected from fresh tree wounds in 1953 and 1954 included representatives from the families Nitidulidae, Ostomidae, Histeridae, Cucujidae, Staphylinidae, Scolytidae, Brentidae, and Curculionidae. The nitidulid species were *Glischrochilus fasciatus* (Ol.), *G. sanguinolentus* (Ol.), *Carpophilus lugubris* Murr., *Carpophilus* sp., *Colopterus truncatus* (Rand), *C. morio* (Er.), *Cryptarcha ample* (Er.), *Stelidota geminata* (Say), *Lobiopa undulata* (Say) and *Epuraea* sp. The ostomid species included only *Tenebroides* sp.; *Platystoma* sp. and *Hister* sp. represented the Histeridae. *Silvanus* sp. was the only cucujid present. There was one staphylinid, *Philonthus* sp.; one scolytid *Monarthrum mali* (Fitch); one brentid, *Arrhenodes minuta* Drury and one curculionid, *Conotrachelus anaglypticus* (Say) represented in these collections.

INSECT TRAPPING STUDIES IN THE FOREST.—Empirical observations in the field soon established the fact that insects, especially nitidulids, are attracted to and begin to feed upon the myclial mats in oak wilt trees as soon as bark cracks appear (Figure 4). These mats have a characteristic, pungent aroma that is attractive to nitidulids and other fungus feeders.

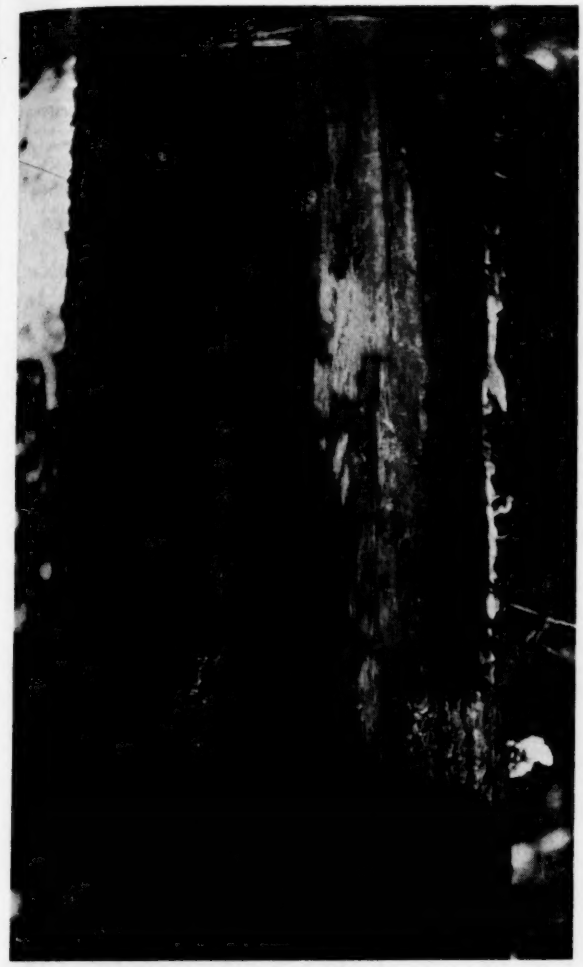

FIG. 3.—Blaze type wound showing a dark fungus-infected area and a light area noninfected, from which the bark had just been removed.

In order to learn more about the effectiveness of natural and artificial baits, substances (naturally derived from naturally occurring fungi) as well as baits formulated from laboratory ingredients were tested to determine their attractiveness to insects commonly found on and in oak

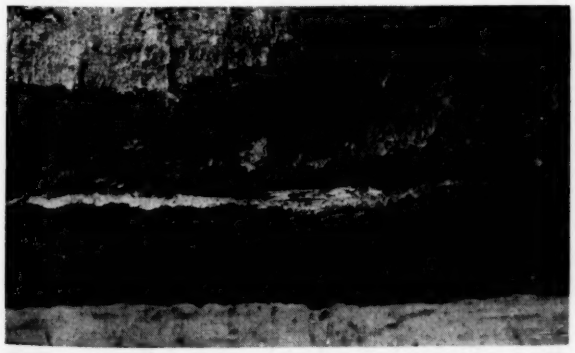

FIG. 4.—Bark crack caused by the pressure cushions of an oak wilt mat.

wilt trees. A series of trapping experiments was started early in the summer of 1952 and was conducted continuously, 12 months each year, until the end of the summer of 1954.

The trapping sites were located in dense woods mainly in the Coopers Rock State Park area remote from dwellings. During the initial phases of these trapping trials the approach was somewhat exploratory with respect to trapping site locations, time of bait exposures, and other variations in collecting procedure. These variations in methods during the summer and winter of 1952 make accurate and dependable comparative studies of the baits impossible for this period.

Starting in the summer of 1953 and continuing without interruption until the end of the summer of 1954, trapping methods and procedures were uniform and the data from these collections were the basis for comparison and statistical analysis. Seven trees were selected as trapping sites; they were located about 50 yards apart along a mountain ridge. Each tree was provided with a wire bait holder (rack) which held seven baits. This rack was nailed to the

FIG. 5.—Insect bait traps at a trapping site.

tree at a point about 6 feet above the forest floor (Figure 5). The rack was shaped to conform to the contour of the tree trunk in a horizontal plane. The individual bait containers were 50-ml. beakers. Each container was provided with a conical, waxed-paper roof which was secured by a rubber band and the opening adjusted so that rain was excluded and insects were prevented from inadvertently falling into the bait. The bait containers were spaced approximately 4 inches apart in the rack around the tree and each bait was in direct competition with the others. One control beaker containing water was included with each series (rack) of competing baits. All baits were in liquid form and served to attract and to trap the insects. The fungus baits were grown in a liquid, glucose—urea medium[3] and the other baits were mixed

[3] For *E. fagacearum:* Glucose 50 gm., Urea 1. gm., $MgSO_4 7H_2O$ 0.5 gm., KH_2PO_4 1 gm., Fe^{+++} +0.2 mg., Mn^{++} 0.1, biotin 5 μg., thiamine 100 μg., distilled water 1 liter, pH 5.5.

For other fungi: Same formula except the following ingredients were also added: Pyridoxine 100 μg./1 and inositol 5 mg./l.

The fungi were cultured in 2800-ml. Ferenbach flasks which were closed with 600-ml. beakers. Each flask contained 1 liter of medium. The filtrates were used as baits when the cultures were from 2 to 3 weeks old.

Table 2.—Coleoptera reared from oak wilt trees.[a]

Adults Collected[b]	Total Number Collected	
	Species	Specimens
SCOLYTIDAE	8	18,050[c]
Pseudopityophthorus pruinosus (Eichh.)		
P. asperulus (Lec.)		
Monarthrum fasciatum (Say)		
M. mali (Fitch)		
Stephanoderes sp.		
Xylosandrus germanus (Bldfd.)		
Xyleborus saxesenii (Ratz.)		
Xyleborus sp.		
STAPHYLINIDAE	9	303
Aleocharinae (5 species)		
Philonthus (2 species)		
Conurus sp.		
Tachinus sp.		
NITIDULIDAE	10	175
Colopterus semitectus (Say)		
C. morio (Er.)		
Glischrochilus fasciatus (Ol.)		
G. sanguinolentus (Ol.)		
Carpophilus lugubris Murr.		
Epuraea (3 species)		
Stelidota geminata (Say)		
Cryptarcha ample Er.		
ORTHOPERIDAE	7	242
Molamba sp.		
Laemophleous (4 species)		
Brontes sp.		
One unidentified genus		
CRYPTOPHAGIDAE	2	175
Atomaria sp.		
One unidentified genus		
COLYDIIDAE	4	138
Bitoma sp.		
Three unidentified genera		
CUCUJIDAE	1	73
Silvanus sp.		
RHIZOPHAGIDAE	2	42
Rhizophagus (2 species)		
CURCULIONIDAE	2	38
Cryptorhynchus pumilis Boh		
Plocamus hispidulus Lec.		
HISTERIDAE	3	31
Platystoma sp.		
Acritus sp.		
Hister sp.		
CLERIDAE	1	23
Phyllobaerus unifasciata (Say)		
MORDELLIDAE	3	8
Mordellistena sp.		
Anaspis sp.		
Catops sp.		
LATHRIDIIDAE	3	7
Corticaria sp.		
Aridius nodifer (Westw.)		
Enicmus aterrimus (Motsch.)		
THROSCIDAE	1	6
Aulonothroscus sp.		
MYCETOPHAGIDAE	3	4
Mycetophagus (3 species)		
CISIDAE	1	1
Ennearthrum sp.		
PSELAPHILDAE	1	1
Unidentified genus		
PLATYPODIDAE	2	2
Platypus compositus (Say)		
Platypus sp.		
CERAMBYCIDAE	1	1
Phymatodes sp. (Say)		
LEIODIDAE	1	2
Anisotoma sp.		
OSTOMIDAE	1	1
One unidentified genus		
BUPRESTIDAE	1	1
Agrilus sp.		
ANOBIIDAE	2	2
Ernobius sp.		
One unidentified genus		
MYCETOPHAGIDAE	1	44
Litargus sp.		
DASCILLIDAE	1	1
One unidentified genus		
CHRYSOMELIDAE	1	1
Epitrix cucumeris (Harris)		
Grand total	72	19,452

[a] 1952, Red Oak, 24" d.b.h. felled, sectioned and placed in rearing cage May 30; collections made twice weekly until August 8. Tree from Centerville, W. Va. 1953, Red Oak, 24" d.b.h. from Morgantown, W. Va. Similarly processed; collections made once a week until August 10.

[b] A few specimens of miscellaneous Hymenoptera, Diptera, Lepidoptera, and Hemiptera were taken but not counted.

[c] 17,794 of these were *M. fasciatum*

with water. The 14 baits used in these trials are identified in figure 6.

The trapping sites were visited for maintenance pur-

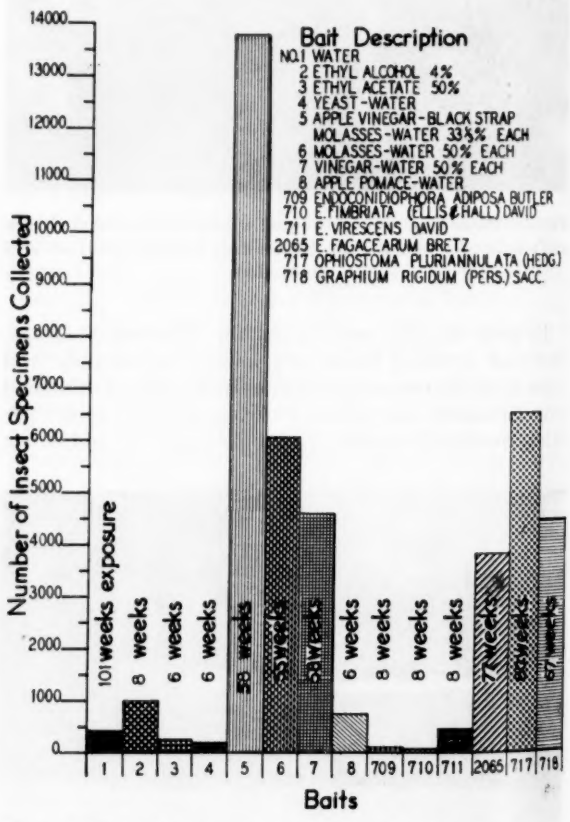

FIG. 6.—Kinds of baits and total number of all insect specimens trapped in each bait from July 17, 1952 continuously through August 19, 1954.

Table 3.—Total numbers of specimens trapped in each of the baits, July 17, 1952 through August 19, 1954.

ORDER	BAITS BY NUMBER[a]														TOTAL
	1	2	3	4	5	6	7	8	2,065	717	718	709	710	711	
Coleoptera	60	507	14	53	889	350	822	98	1,135	1,328	816	14	2	32	6,120
Hymenoptera	143	5	3	5	638	686	179	19	1,196	967	1,087	9	7	13	4,937
Lepidoptera	39	4	1	4	424	534	183	8	421	672	520	1	1	3	2,815
Diptera	165	494	217	137	11,829	4,686	3,540	583	1,209	3,813	2,114	60	69	391	29,307
Orthoptera	0	2	0	1	25	20	3	0	12	13	8	1	0	1	86
Mecoptera	0	0	0	0	1	0	0	0	3	2	1	0	0	0	7
Collembola	6	1	0	0	0	0	0	0	2	9	27	0	0	0	45
Neuroptera	0	0	0	0	8	4	1	0	8	28	37	0	0	0	86
Homoptera	2	1	0	1	8	0	3	0	0	1	2	0	0	0	18
Hemiptera	1	0	1	0	1	1	0	0	1	5	4	0	0	0	14
Diplopoda	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2
Chilopoda	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Arachnida	3	2	4	0	6	3	6	0	3	7	7	1	0	1	43
Acarina	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Totals	419	1,017	241	201	13,829	6,284	4,737	708	3,990	6,847	4,623	86	79	441	43,502

[a] See figure 6 for description of baits.

poses at weekly intervals when the trapped insects were removed and fresh baits provided for each rack. The position of each bait was changed in the rack in a random manner when the baits were renewed. All insects collected were subsequently sorted to order or family rank and the coleopterous specimens were identified to species when possible.

The records of the total number of specimens caught by all trapping methods during the entire trapping program are presented in table 3. The results of the standardized trapping trials for 1953 and 1954, (Nitidulidae) using seven baits, are presented in table 4 and similar data for the other families of the order Coleoptera are given in table 5.

In comparing the effectiveness of the different baits it is evident that the fungus baits are the most attractive to nitidulids (Fig. 7). This difference is significant when the data are analyzed. In comparing the effectiveness of fungus and artificial baits in attracting Coleoptera the data used represent the trapping results from 55 seven-day exposures of each bait. All data were treated by the analysis of variance, square root transformation method. The attractiveness of the fungus baits for Nitidulidae (36.79 F value for comparison of means) and Pyrochroidae (11.84 F value for comparison of means) was significant at the 1% level and for Brentidae (5.83 F value for comparison of means) it was significant at the 5% level. The pooled mean numbers of nitidulid specimens trapped in fungus baits was 9327.7 and in artificial baits, 2890.1. These comparable figures for the family Pyrochroidae were 422.3 and 81.2 respectively. Comparable figures for Brentidae were 149.1 and 139.7. It is believed that the predaceous Pyrochroidae were attracted to the fungus baits because they found more insects for food in these.

The nitidulids were the most abundant of all insects collected in the baits except certain Diptera (Drosophilidae). Dorsey & Carson (1956) have reported elsewhere the Drosphilidae collected during this same trapping experiment. There were five genera and 17 species of Nitidulidae attracted by the various baits (Table 4).

Table 4.—Species and number of specimens of Nitidulidae trapped in each of the baits during 55 weeks of exposures, August, 1953 to August 19, 1954.

SPECIES	BAITS BY NUMBER[a]							
	1	5	6	7	2065	717	718	Total
Gliischrochilus confluentus (Say)	2	264	52	125	101	139	86	769
G. fasciatus (Ol.)	4	16	26	51	199	69	94	459
G. sanguinolentus (Ol.)	2	19	10	50	111	62	85	339
G. sanguinolentus var. *rubrimaculatus* Reih.	0	0	0	0	5	1	1	7
G. quadrisignatus (Say)	0	6	5	14	17	6	7	55
Cryptarcha ample Er.	2	9	49	29	173	92	78	432
C. strigulata Pars.	2	4	11	9	13	8	19	66
Epuraea sp.	0	17	2	24	24	4	19	90
Carpophilus corticinus Er.	0	2	0	13	3	2	0	20
C. lugubris Murr.	0	9	6	23	38	17	16	109
Carpophilus sp.	0	10	9	8	12	14	13	66
C. hemipterus (L.)	0	1	0	0	1	0	0	2
Colopterus truncatus (Rand)	0	0	1	9	2	0	1	13
C. semitectus (Say)	0	0	0	2	8	0	1	11
C. maculatus (Er.)	0	1	0	0	4	0	4	9
C. morio (Er.)	0	0	2	0	2	0	0	4
Totals	12	358	173	357	713	414	424	2451

[a] See figure 6 for description of baits.

Table 5.—Families of Coleoptera and number of specimens trapped in each of the baits during 55 weeks of bait exposures, August 1953 to August 19, 1954.

FAMILY	BAITS BY NUMBERS[a]							
	1	5	6	7	2065	717	718	Total
Nitidulidae	24	193	106	307	560	666	424	2280
Ostomidae	0	1	1	3	4	3	1	13
Scarabaeidae	0	0	0	0	1	1	1	3
Curculionidae	0	0	0	0	2	1	2	5
Cleridae	4	8	4	6	1	23	1	47
Elateridae	1	2	3	0	0	4	0	10
Erotylidae	1	1	0	1	4	1	0	8
Staphylinidae	0	0	2	9	7	3	1	22
Scolytidae	1	1	1	0	0	2	1	6
Histeridae	0	3	0	1	2	0	1	7
Cerambycidae	2	8	9	8	9	24	8	68
Carabidae	0	0	1	0	0	1	1	3
Mordellidae	2	4	0	6	0	8	7	27
Brentidae	0	2	4	2	2	3	1	14
Pyrochroidae	2	6	11	2	22	39	15	97
Cucujidae	0	2	0	0	0	0	0	2
Silphidae	1	1	1	2	17	36	32	90
Chrysomelidae	0	0	2	0	1	1	0	4
Lampyridae	0	0	0	0	2	4	1	7
Buprestidae	0	0	1	0	0	0	0	1
Grand total								2714

[a] See figure 6 for description of baits.

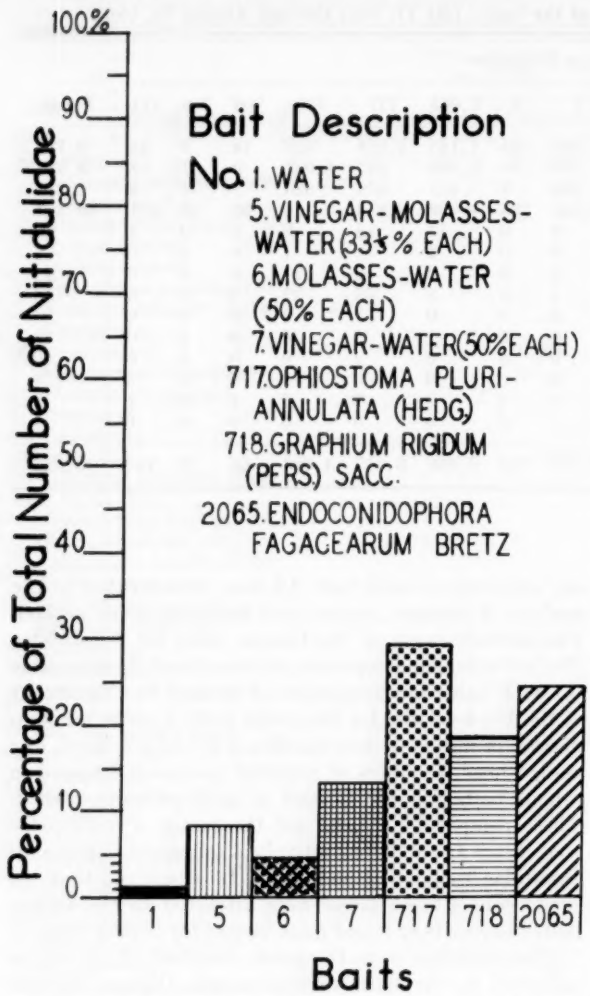

FIG. 7.—Description of the baits and the percentage of the total number of Nitidulidae trapped in each during 1955.

Four species (*Glischrochilus sanguinolentus*, *G. fasciatus*, *G. confluentus* and *Cryptarcha ample*) constituted more than 80% of the total number of nitidulids collected for 2 consecutive years. Trapping records pertaining to these particular species were recorded for 55 seven-day bait exposures and are presented in figure 8. The artificial baits were Nos., 1, 5, 6 and 7 and the fungus baits used were Nos. 2065, 717 and 718 (Figure 7). The fungus baits were almost twice as effective in attracting these nitidulids as were the artificial baits. During the colder months (December through March) freezing apparently had an adverse effect on the fungus baits and they were not as good attrahents as the artificial baits. However, during this period only *G. confluentus* was present in numbers and this species may prefer the aromas of artificial baits. By the time warm weather arrived this species had practically disappeared so the efficaciousness of fungus baits in attracting this insect was not ascertained.

It is believed that the presence of carnivorous and necrophagous insects in the various baits can be explained by the fact that large quantities of their preferred food were concentrated in the small areas of the bait containers. If the fungus aromas attracted them it is possible that these aromas are associated with food in their special way of life.

LIFE AND SEASONAL HISTORY STUDIES OF NITIDULIDAE.—The nitidulids have been correctly designated as sap and fungus feeding bettles. In these studies they were always the predominating insects on and in moist tree wounds. They were also the most abundant forms in fungus communities, under tree bark, such as the oak wilt mycelial mats. These beetles and their larvae feed on the fungi commonly associated with this type of environment. Several species of *Carpophilus*, *Glischrochilus*, *Colopterus* and *Epuraea* have been observed, both in the field and laboratory, to feed and to develop satisfactorily on fungi including the oak wilt fungus. Curl (1955) also observed that representatives of those genera (except *Epuraea*) feed destructively on the oak wilt fungus under laboratory conditions.

Nitidulids react quickly, in general, to various stimuli. When disturbed in their natural environments they rapidly disappear by hiding in cracks or by feigning death and dropping to the ground or by flying away. The adults have typically flattened bodies and are well adapted for inhabiting small crevices between the bark and sapwood of wounded trees. All of the species observed in these studies are capable of rather strong flight. Morris *et al.* (1955) recovered two tagged nitidulids 1 mile from the point of release within a 24-hour period. Similar experiments were conducted by the authors in 1954, but the numbers of nitidulids available at the time of the tests were too few to permit valid conclusions to be drawn from the results obtained. In these tests radioactive phosphoric acid was incorporated in the medium upon which the fungus was grown. The nitidulids were permitted to feed on the fungus before their release for distribution studies. It was found that this method of tagging was more efficient than simple immersion of the insects in radioactive solutions.

The olfactory sense of this group of bettles is acute. The strong, characteristic aroma of oak wilt mycelial mats readily attracts nitidulids and certain other fungus feeders. In April, 1952 a large oak wilt tree was felled and sectioned. One of the trunk sections contained a large, well developed mycelial mat which was exposed by a bark crack. This section was placed outside of the laboratory building on a cement driveway. A square section of bark covering the mat was carefully excised so that it could be removed from and replaced over the mycelial mat. For the purpose of collecting insects this piece of bark was removed twice daily (morning and late afternoon) for a week. Each morning following the afternoon collection, the nitidulids were present in numbers comparable to those in the preceding afternoon collection. This mat did attract nitidulids and the nearest concentration of trees or woods was at least ¼ mile away. They apparently came to the mat both at night and during the day about equally well in this particular period of observation.

Nitidulids are evidently widely dispersed in the forest and when an attractive environment is made available to them they soon utilize it. The forest inhabiting species travel both at night and during the day in this area. Other nitidulids apparently have different habits; Barnes

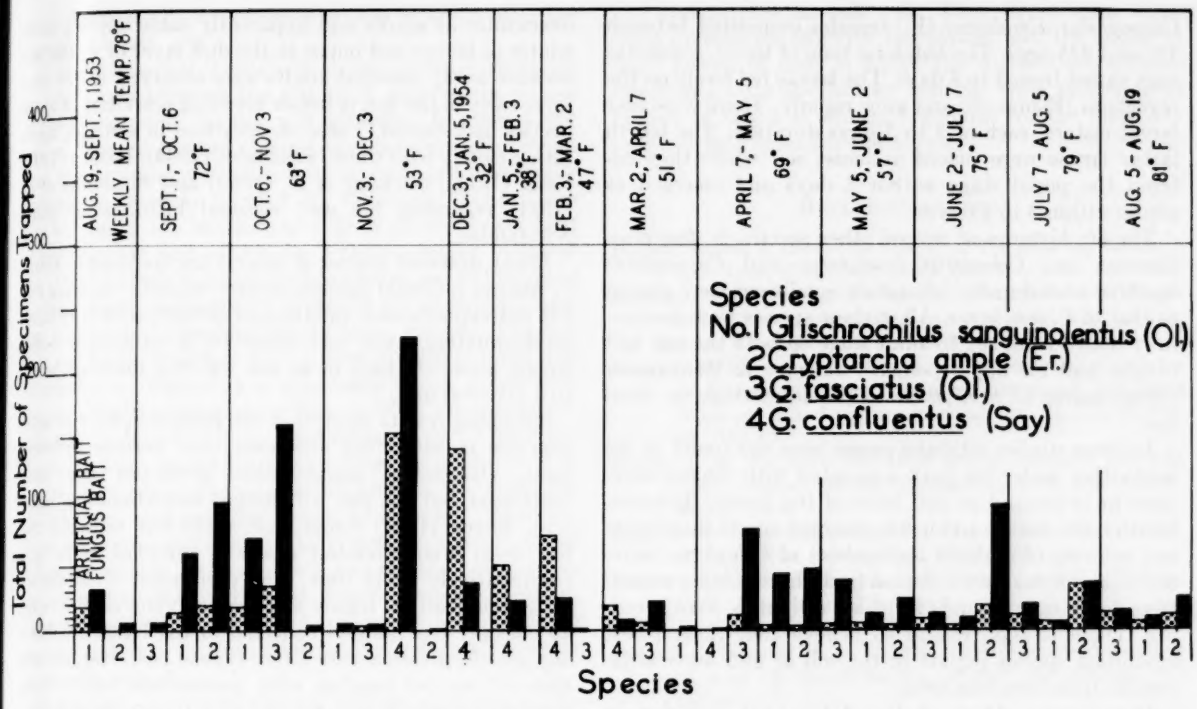

FIG. 8.—Bait preference (fungus and artificial baits) and seasonal distribution of the four most prevalent nitidulid species as shown by the numbers trapped in the two types of baits from August 19, 1953 to August 19, 1954.

& Kaloostian (1940) observed that *Carpophilus hemipterus* flies mainly during daylight hours.

In West Virginia nitidulids were trapped during every month of the year for 3 years. The species pattern varied with the seasons, but active nitidulids were always present. *Glischrochilus fasciatus* and *sanguinolentus* were present and active at all times of the year (Fig. 8) except when the mean weekly temperatures were around 32° F. *G. confluentus* was the predominant species during cold weather and frequently was collected frozen in baits; later the specimens would thaw and become active. This characteristic seemed to be typical of the forest inhabiting nitidulids; low temperatures inactivate them, but they soon resume activity when the temperature rises above freezing. *G. confluentus* was the only species which could be readily collected during January, February and March. *G. fasciatus* is an early spring species which begins to appear in numbers in early April. Later in April *G. sanguinolentus* becomes numerous and about the first of May *Cryptarcha ample* appears. *C. ample* is the most abundant hot weather species. The other nitidulids listed in the collection records are most active in spring, summer and early fall. In West Virginia the periods of maximum nitidulid populations are in September, October and early November and to a lesser degree in April, May and early June. The most favorable temperature conditions apparently are mean weekly temperatures of about 50° F. High summer temperatures with lowered humidity conditions reduced the number of nitidulids which were trapped.

Life and seasonal histories of nitidulids were studied under field and insectary conditions. Beginning in June 1953 *Colopterus semitectus* was observed more carefully, in this respect, than any other species. Adults of this species, congregated in a blaze wound on an oak within 2 days after the wound was made. On the third day nitidulid eggs were found on the inner surface of the loosened bark (Fig. 9). Three days after oviposition the eggs began to hatch. For various reasons, it was necessary to continue these life history studies in the insectary. Adult *C. semitectus* beetles were collected from this same tree wound and were placed on cultures of the oak wilt pathogen on agar plates. Feeding and mating began almost immediately. The females were isolated on similar plates (cultures) and an average of 72 eggs was produced by each female. Balzer (1942) found that individual

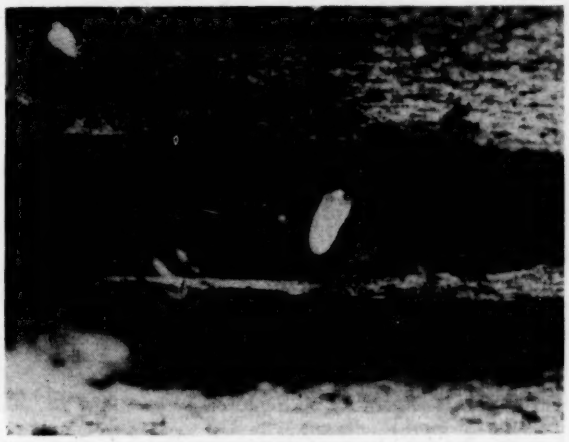

FIG. 9.—Nitidulid eggs in a natural habitat in a tree wound under loosened bark.

Carpophilus dimidiatus (F.) females oviposited between 175 and 225 eggs. The hatching time of the *C. semitectus* eggs varied from 3 to 5 days. The larvae fed freely on the mycelium (Figure 10) and grew rapidly. There were four larval instars, each of 3 to 5 days duration. The fourth instar larvae were placed in moist soil where they entered the pupal stage within 2 days and emerged as adults within 3 to 4 days.

The life histories of several other species, *G. fasciatus*, *Epuraea* sp., *Colopterus hemipterus* and *Carpophilus lugubris*, reared under laboratory conditions were similar to that of *C. semitectus*. All of these species were successfully reared from egg to adult stage on both the oak wilt fungus and on apple pomace. Jacquet & Bonnamour (1929) reared *C. hemipterus* to the adult stage on dried figs.

In these studies nitidulid pupae were not found in the field either under the bark associated with fungus infections or in the soil or duff layer of the forest. However, fourth instar larvae and newly emerged adults (hardening and coloring of cuticula incomplete) of *Colopterus morio* and *C. semitectus* were collected from the duff layer around trees containing wounds in which nitidulids were breeding. There is good reason to believe that these forest inhabiting species pupate in the soil or duff layer adjacent to their breeding sites.

DISCUSSION.—Most of the Nitidulidae included in these studies are apparently, by preference, sap and fungus feeders. They quickly respond to the stimuli of sap and fungus aromas emanating from moist wounds on living trees and upon arrival at the site soon begin to feed and mate. They have a comparatively short life history (21 to 25 days) and several generations are produced during the year in West Virginia. The nitidulids overwinter as adults and apparently some species overwinter as larvae and pupae in the duff layer or in the soil because newly emerged adults were observed in the duff layer early in the spring before breeding activities of these beetles had started. These observations in general, agree rather closely with those of Schmidt (1935), Balzer (1942) Essig (1915), Stickney *et al.* (1950) and Simmons *et al.* (1931) regarding life and seasonal histories of other Nitidulidae.

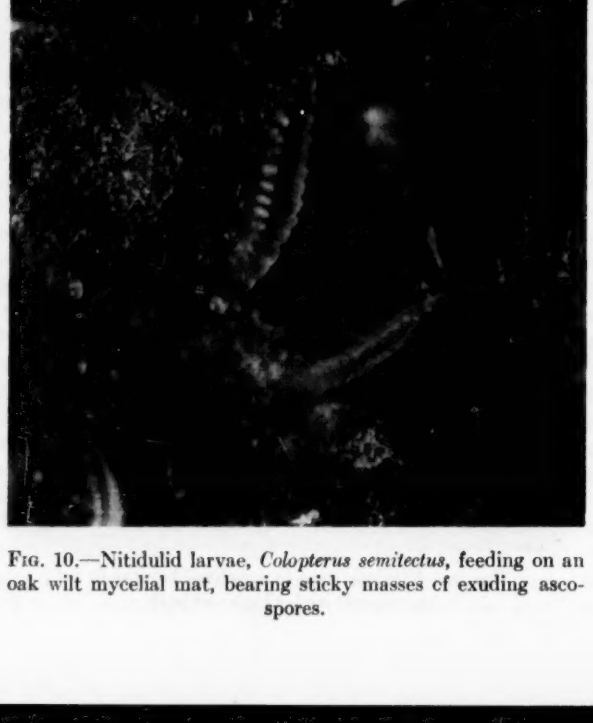

FIG. 10.—Nitidulid larvae, *Colopterus semitectus*, feeding on an oak wilt mycelial mat, bearing sticky masses of exuding ascospores.

Many different species of insects are commonly found in natural nitidulid colonies in tree wounds. As many as 175 individual insects (adults and larvae), with nitidulids predominating, have been observed in an area 8 inches square under the bark of an oak wilt tree on or adjacent to a mycelial mat.

Trapping results show that the fungus baits are more effective in attracting nitidulids than certain artificial baits. *Ophiostoma pluriannulata* attracted the most specimens, but the oak wilt fungus was almost as effective. Boyce (1954) found that nitidulids released 500 feet away would reinfest oak wilt mycelial mats in a comparatively short time. The preference of nitidulids for fungus baits is highly significant. None of the other 23 coleopterous families collected in the baits exhibited any specific preference for either type of bait. Representatives of several families with predaceous habits were apparently attracted to the baits by the easily procured insects trapped in them.

Nitidulids were usually associated in nature with the fungi *Ophiostoma pluriannulata*, *Graphium rigidum* and with *Endoconidiophora fagacearum*. Because of the sticky nature of the matrix in which the spores of *O. pluriannulata* and *G. rigidum* are produced they are not disseminated by wind, but an insect coming in contact with the sticky matrix could easily become contaminated. It is evident that nitidulids are largely responsible for contaminating tree wounds with these non-pathogenic fungi, since in some fungus-infected wounds only nitidulids were observed to be present. It is significant that in attempting to isolate the oak wilt pathogen from adult nitidulids these fungi were found to be among those most frequently cultured. These same fungi have also been found commonly growing adjacent to or intermingled with an oak wilt mycelial mat that was infested with nitidulids which had gained access to the mat through a bark crack. These observations further support the conclusion that the nitidulids attracted to the mat carried with them spores of *G. rigidum* and *O. pluriannulata* which subsequently became established there.

It has been demonstrated by a number of workers that these beetles also disseminate spores of the oak wilt pathogen. Jewell (1954) demonstrated that conidia of the oak-wilt fungus ingested by nitidulids can pass through the intestinal tract and are subsequently eliminated in a viable condition. Yount *et al.* (1955) recovered the oak wilt pathogen from the external surface of the adult nitidulids.

The fact that nitidulids are quickly attracted to fresh wounds and to fungi was established empirically in the forest around tree wounds, natural fungus infections and at special bait stations. Zuckermann (1954) observed that a fresh wound in an oak serves best as an infection

court for oak wilt if inoculated within 24 hours after the wound is made.

It is obvious that there exists a relationship of mutualistic symbiosis between the nitidulids and certain species of fungi including the oak wilt pathogen. In areas where oak wilt is not present *Ophiostoma pluriannulata*, *Graphium rigidum* and several species of yeast are universally present in wounds infested by nitidulids. These fungi and the moist sap in which they grow constitute the food of both larvae and adults. Since the spores, by virtue of the sticky matrix in which they are borne, are not adapted to wind dissemination they probably are dependent upon the insects for dissemination to fresh wounds. The oak wilt pathogen which is transmitted both externally and internally by nitidulids is apparently a new member in the symbiotic association, at least in West Virginia where oak wilt is found only in local areas scattered over the state. As time passes, and in the absence of an effective control procedure, the association between the nitidulids and the oak wilt pathogen might be expected to become more and more widespread. An increase in the amount of wilt comparable to that which has already occurred in certain areas of the midwest would probably develop. This probability is an added reason for attempting to control oak wilt in the eastern states before it becomes more widespread.

SUMMARY AND CONCLUSIONS.—Representatives of the family Nitidulidae were the most numerous of all insects attracted to fresh tree wounds and to oak wilt mycelial mats in dying oak wilt trees. The nitidulids collected in traps in the forest preferred fungus type baits to artificial baits. *Ophiostoma pluriannulata* and *Endoconidiophora fagacearum* were respectively the two most effective attrahents. Some species of Nitidulidae are active in the forest all of the year; species predominance varies with the season. Maximum populations of nitidulids occurred during the early spring and fall when the mean weekly temperature was approximately 50° F. The life cycle of certain nitidulid species studied is comparatively short (21 to 25 days) under optimum conditions. In West Virginia they produce at least three generations each year. Eggs are deposited under the bark in moist wounds that consistently become overgrown with fungi, the most prevalent of which are *Ophiostoma pluriannulata* and *Graphium rigidum*. They are also deposited on the mycelial mats of *E. fagacearum*. The eggs hatch in 3 days and there are four larval instars. The larvae feed on the fungi and when ready to pupate apparently fall to the ground and enter the duff layer. The species observed over winter as free living adults and some probably as pupae in the duff layer.

A mutualistic symbiosis exists between the nitidulids and their associated fungi among which *E. fagacearum* is a new member in West Virginia. As time passes, the association of the nitidulids and the oak wilt pathogen is likely to progress with a corresponding increase of the disease. For this reason, prompt and immediate efforts to control oak wilt are adequately justified.

Because of feeding and breeding habits, relatively high reproductive potential, flight and dispersal characteristics, quick response to stimuli and prevalence of some species during all seasons of the year, the nitidulids meet most of the requirements for an effective vector of a fungus disease such as oak wilt. Their dependence upon fresh wounds for effective inoculation and the apparent short period of time in which the wounds are suitable infection courts are factors limiting their efficiency as vectors of oak wilt and may account for the relatively slow overland spread of the disease.

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Effect of Insecticides on Corn Root Growth[1,2]

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The use of soil insecticides in agriculture is limited by several factors. Among these are effectiveness, cost, toxicity to man and a factor of prime importance which we may call selective toxicity. A material which is selectively toxic in the useful sense is one which kills or injures unwanted species of plants or animals without causing damage to the useful forms. A good soil insecticide should have qualities of selective toxicity in addition to effectiveness and relative low cost.

Aldrin, BHC and heptachlor have been widely used as soil insecticides especially for the control of the several species of corn rootworms (*Diabrotica* spp.). Although the general phytotoxic effects of BHC have been recorded for a large series of plants, little information is available concerning the specific phytotoxic effects to corn caused by these insecticides. The purpose of this study, therefore, was to determine the relative phytotoxic effects to the corn plant caused by the above mentioned toxicants when applied to various culture media.

The chief disturbance caused by BHC is that of inducing cytological abnormalities in the meristematic region of the root tip. Kostov (1948) reported that BHC suppressed root, stem and colioptyle development of gramineous plants. Such abnormal growth produced thickening of these organs, especially the growing point. Doxey & Rhodes (1951) working with rye noted that the effects of BHC were similar to the effect of colchicine and other mitotic poisons by inducing polyploidy and causing enlarged nuclei of irregular shape. Simkover & Shenefelt (1952) detected microscopic swelling of root tip of Norway pine seedlings treated with BHC at the rate of 1.0 pound gamma isomer per acre. Cytological malformations were confined to the meristematic region of the root tips. Root injury of the pine seedlings when planted in white sand was noticeable following a rate of application as low as 8 ounces of gamma isomer of BHC per acre whereas rates as low as 1.0 p.p.m. suppressed growth. Soil particle size and pH little influenced the capacity of BHC to suppress growth.

Scholes (1953) reported that the alpha and beta isomers of BHC at rates of aplication up to 4.0 pounds per acre had no effect on the growth rate or development of onion seedlings although some cytological abnormalities were present. The delta and gamma isomers were comparable in causing polyploidy at rates of 3.2 and 4.0 pounds per acre. At low dosages (0.8 or 1.6 pounds per acre) inhibition of spindle formation occurred causing delayed or incomplete chromosome separation.

Fulton (1946) observed injury to field corn treated with 5 to 7 gms. of 8 per cent gamma isomer of BHC per hill. Plants so treated averaged less than half the size of control plants. In a similar experiment at another location no damage occurred. Grayson & Poos (1947) reported that 100 pounds of 30% gamma isomer of BHC per acre on sandy loam soil low in organic matter completely killed corn soon after germination. Fleming & Maines (1953) found that corn growth was retarded when treated with lindane at a rate as low as 2.0 pounds per acre.

NUTRIENT CULTURE STUDIES.—*Methods and Materials.*—Corn seeds were germinated in a rag doll and when the coleoptile was approximately 1 inch long the plants were transferred to 2-quart Mason jars which were used as culture vessels. Wooden blocks having $\frac{3}{4}$-inch holes in the centers to hold the growing plants were inserted in the jar openings. Each jar was covered with several layers of dark paper and fitted with an aerating tube. Additional artificial light was used to supplement the available greenhouse light. Nutrient culture studies as well as soil and sand culture experiments were conducted at a thermostatically controlled temperature of 75° F. $\pm 3°$.

The vessels were filled with a complete nutrient solution (Hoagland and Arnon solution No. 1, 1950) having a pH of 6.7. Small amounts were added periodically to keep the volume constant. Additional iron in the form of iron citrate was added as required. Arbitrarily, the 2-quart jars were considered the equivalent of $\frac{1}{2}$ square foot of surface area and the aliquots of insecticide in emulsion form were added and mixed accordingly.

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[2] This investigation was supported in part by research grants from Shell Chemical Corporation, Denver, Colorado, and Velsicol Corporation, Chicago, Illinois.

Table 1.—Average plant height and dry root weight of corn plants 48 days following planting in nutrient culture.

Treatment	Height (Cm.)		Weight (G).	
	0.5 lb./A.	1.0 lb./A.	0.5 lb./A.	1.0 lb./A.
Aldrin	134.10	109.50	2.12	1.77
BHC	82.37	61.25	1.90	0.78
Heptachlor	122.75	103.25	2.78	2.59
Check	130.50	138.37	3.05	3.05
L.S.D. at 5% level	—	—	ns	1.80
at 1% level	18.65	28.31	ns	—

Aldrin, BHC and heptachlor were compared at application rates of 0.5 and 1.0 pound per acre. This was calculated to be 7.5 and 15 p.p.m. respectively. The insecticides used were emulsifiable concentrates containing 23.1% aldrin, 27.5% BHC (11% gamma) and 23.41% heptachlor. The treatments were replicated four times and the entire experiment was repeated twice.

Results.—The experiment was terminated 48 days following placement of the plants in the nutrient-insecticide solution. Table 1 shows the averge plant height and dry root weight at that time.

An inspection of table 1 indicates the severe phytotoxicity caused by BHC in nutrient solutions. This is evident both in plant height and dry root weight. Even under these extreme conditions aldrin- and heptachlor-treated plants compared favorably with the check plants (Fig. 1).

Sand Culture Studies.—*Methods and Materials.*—In the sand culture experiments the corn was germinated and grown in flats 1 foot square and 4½ inches deep. The flats were coated on the interior surfaces with beeswax to prevent unequal water loss during the germinating and growing period. The sand was treated and thoroughly mixed with emulsifiable aldrin, BHC and heptachlor prior to planting. The pH of the mixture was 6.7. Rates of application were 0.5 and 1.0 pound actual toxicant per acre. The seeds were planted at a uniform depth of 2 inches, each flat containing 3 rows of 10 plants each. The treatments were replicated nine times.

Results.—One week following germination the plants were washed clean from the sand and the four longest roots from each plant were measured to the nearest half centimeter. Average root lengths are given in table 2.

BHC proved to be the most toxic and the only toxicant which produced consistent damage in sand (Table 2).

Table 2.—Average length of the four longest roots per corn plant[a] one week following germination in a sand culture.

Treatment	Average Root Length (Cm.)	
	0.5 lb./A.	1.0 lb./A.
Aldrin	11.51	11.68
BHC	9.19	4.46
Heptachlor	12.54	12.33
Check	12.71	12.68
L.S.D. at 5% level	2.00	1.60

[a] Each figure represents measurements for 90 plants.

In addition to shorter root length, BHC-treated plants had considerably fewer secondary roots and root hairs. The roots of aldrin-treated plants were not significantly shorter than the check roots. Heptachlor-treated plants possessed roots which compared favorably with the check or control plants, indicating practically no phytotoxic or inhibitory effects at the observed rates.

Soil Culture.—Soil culture experiments were conducted in the same manner as the sand culture tests. The media used here had a pH of 6.25 and consisted of ⅗ soil, ⅕ sand and ⅕ dried peat moss by volume. It was so formulated to determine the effect of aldrin, BHC and heptachlor on root growth in an organic type soil. The insecticides were applied at rates of application of 0.5, 1.0, and 2.0 pounds per acre and thoroughly mixed with the soil prior to planting. Root length measurements were taken as described in the sand culture experiments.

Table 3.—Average length of the four longest roots per corn plant[a] one week following germination in a soil culture.

Treatment	Average Root Length (Cm.)		
	0.5 lb./A.	1.0 lb./A.	2.0 lbs./A.
Aldrin	13.31	12.62	13.12
BHC	11.97	13.35	13.60
Heptachlor	14.61	14.44	14.95
Check	13.26	14.13	14.03
L.S.D. at 5% level	1.03	—	—
at 1% level	—	1.10	0.88

[a] Each figure represents measurements for 90 plants.

The results in table 3 indicate a more confused picture than the outcome of the nutrient and sand culture experiments. At the 0.5 pound rate of application only the BHC-treated plants possessed roots which were significantly shorter than those of the check plants, while the roots from the heptachlor-treated plants were significantly longer. At the rates of 1.0 and 2.0 pounds per acre, the aldrin-treated plants were found to have significantly shorter roots. At 2.0 pounds per acre the roots from heptachlor-treated plants were significantly longer. The average root length for the BHC-treated plants was always less than the check average while the root length of heptachlor-treated plants exceeded the check at all treatment levels.

Germination records for corn plants grown in sand or soil previously treated with aldrin, BHC or heptachlor at rates of 0.5 or 1.0 pound per acre indicated no reduction in germination rate with any of the soil insecticides used.

Field Tests.—During the spring and early summer of 1954 corn plants were removed from treated plots in the field for root length measurements. Because rootworm larvae had fed more extensively on the roots of the control plants, an accurate contrast in length with the roots of chemically treated plants was not possible. However, root length measurements determined from plants in replicated plots treated with aldrin, BHC and heptachlor indicated that in three out of four plots the heptachlor-treated roots were longest. BHC-treated plants compared favorably with plants treated with aldrin, but possessed

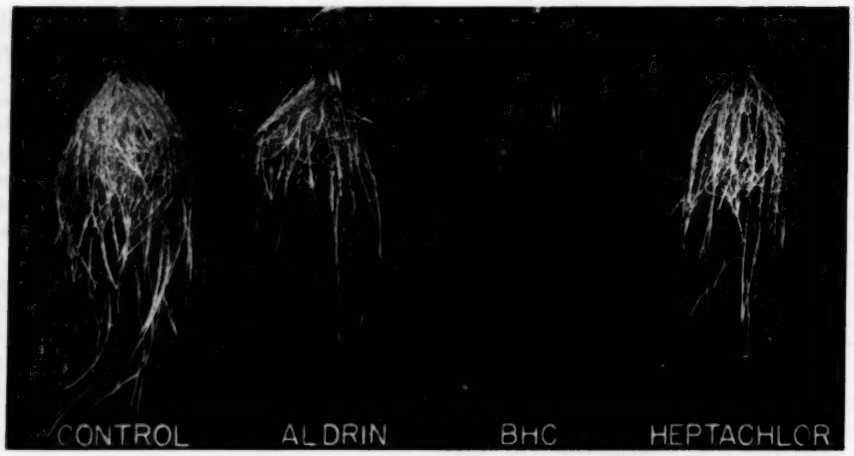

FIG. 1.—A representative series of corn roots grown in nutrient culture at an insecticide concentration of 15 p. p. m. Note the decrease in the numbers of secondary roots on the BHC-treated plants.

roots slightly shorter in length. Three weeks following planting, heptachlor-treated plants averaged 10 grams more in weight than plants from the untreated or the other chemically treated plots.

DISCUSSION AND CONCLUSION.—It is apparent from the data presented that the phytotoxic effects of BHC on corn root growth are much more pronounced in nutrient and sand culture than those of aldrin and heptachlor. Heptachlor appears to be non-inhibitory in all cases and appears to possess some stimulatory properties. Fleming & Maines (1953) however, reported retardation of corn growth when treated with 6.0 pounds heptachlor per acre. Aldrin compares favorably with heptachlor and does not inhibit growth under nutrient or sand culture although slight root growth inhibition did occur in soil at rates exceeding 0.5 pound per acre.

Two types of abnormality resulted from BHC treatment to the corn plant. The first type manifested itself in the enlargement of the root tip region resulting in "clubbing" and much shortened primary roots. Microscopic examination of crushed root tip sections revealed cytological abnormalities similar to those reported by Kostov (1948). The second type of abnormality resulting from BHC treatment was evident from the large reduction in the numbers of secondary roots and root hairs.

Several exploratory experiments conducted by the author indicated that damage from gamma isomer treatment was less than that resulting from BHC exposure. The additional injury observed with the BHC treatments may have resulted from the toxic effects of the delta isomer. This conjecture is supported by the fact that Simkover & Shenefelt (1952) reported that the delta isomer of BHC produced growth inhibition of Norway pine seedlings at lower concentrations than other isomers. Also, Scholes (1953) working with onion (*Allium cepa*) and strawberry (*Fragaria vesca*) indicated that the effects of the delta and gamma isomers were comparable.

The explanation for the reduced phytotoxic effects on root growth in heavier organic type soils is not clear. The range in pH values was narrow and this variation was probably not responsible for the differences in root growth. Simkover & Shenefelt (1952) indicated that pH and soil particle size little influenced the capacity of BHC to induce root malformations. Foster (1951) observed differences in response to DDT when the plants were grown in muck and mineral soils similar to those reported here with BHC. He suggested that the lower phytotoxicity of DDT in muck soils as contrasted to mineral soils may be due to the adsorption of the toxicant by the organic colloids.

Although not significant, there appears to be a growth stimulating effect of heptachlor in the soil. A direct affect on growth processes or enzyme systems does not seem likely since the phenomenon occurred in soil culture only. No stimulation was noted in nutrient or sand culture. It appears that something relating to the soil complex may be responsible for this stimulation. Heptachlor, in contrast to the other toxicants tested, may offer control of some of the soil microflora and -fauna. Also, it may function in some manner in making minerals more available to the plant.

SUMMARY.—The effect of BHC, aldrin and heptachlor on corn root growth was observed in nutrient solutions, sand and soil culture.

BHC treatments in nutrient solution and sand culture resulted in shortened and enlarged primary roots along with a considerable reduction in the number of secondary roots and root hairs.

Heptachlor treatments resulted in plants having roots which compared favorably with the controls whereas aldrin treatments showed only limited damage.

No reduction in germination rate was observed with any of the soil toxicants tested.

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Control of Billbugs on Corn in South Carolina[1]

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For many years corn fields in the United States have suffered from the activities of billbugs. Two of the several species of *Calendra* found on corn in the Southeast, *C. maidis* (Chitt.) and *C. callosa* Oliv. (Blatchley & Leng 1916), occur in such numbers as to be responsible for more loss of stand than any other corn insect in the area.

The adults feed at the base of seedling corn in the spring, and as a result the field has partial to complete loss of stand in an area ranging from a quarter of an acre to 35 acres or more. An injured plant may die, produce suckers, or be weakened to the point that it produces no ears. Eggs are laid in the stalk; feeding larvae reduce both the vitality of the stalks, and the yield, and develop into the adults that will look for corn to feed upon the next year.

Billbugs originally infested cattails, rushes, and related plants growing in bottom land, marshes, and swampy areas (Satterthwait 1919). When these areas were cleared and drained, the first crop to be planted was usually corn. The billbugs transferred their feeding to this crop, whose cultural schedule matched the needs of the billbugs perfectly. Over wintered adults start to emerge from stubble of the previous crop about the time most corn begins to come up, and they continue to emerge over a 2- to 3-week period, depending on soil temperature. Although winged, corn billbugs seldom fly, but crawl over the ground in search of food. Under favorable conditions, they can migrate ¼ mile or more.

Feeding takes place at the base of the stalk. The insect grasps the stalk with its legs, and, with the head down, the "bill" gouges out slits in the outer layers of leaf so that it can feed on the tender inner leaves. The tip of the abdomen is usually visible, protruding from the ground. In many instances, however, the whole insect has burrowed down out of sight at the base of the plant.

Considerable movement takes place as the billbug kills one plant and searches for another. Most of this movement occurs at night, and each time the insect stops to feed or to rest until the next night, it burrows into the soil, presumably to escape the heat and drying effects of the sun. The amount of damage caused by the adult during feeding depends upon the size of the plant and the number of billbugs feeding on it. A small plant may be killed by the feeding of a single beetle, while a slightly larger plant will have the bud killed and then send out unproductive suckers. A still larger plant may continue to grow, but be all twisted and never reach bearing size. Other plants may continue to grow normally, with only a few rows of holes across the leaves to show that they had been injured.

Oviposition starts about 10 days after the first feeding. Eggs are deposited singly in slits similar to feeding slits. About 200 eggs may be laid by each female, with each stalk capable of supporting 1 to 6 larvae to maturity. The eggs hatch into off-white, legless, brown-headed grubs which spend the next couple of months feeding on the tissue in the center of the stalk below ground or just above the ground level. Damage from the larvae results in lower productivity of the plant. If several larvae develop in a single stalk, chances of producing an ear are very slight. These plants are stunted, and are easily distinguished from those having only one or two larvae, or none at all. Pupation in the "tap root" occurs during August, and, in South Carolina, teneral adults may be found in the base of the stalk by mid-September. These adults remain in the below-ground portion of the stalk until the following spring, except for a few that move out and are found within a couple of inches of the stalk.

None of the attempts to control corn billbugs were successful with the materials available prior to the development of the hydrocarbons (Metcalf 1917, Cartwright 1929). It was known that proper rotation would keep the infestation at a low level, but such rotation was not possible on many farms, especially those too small to avoid migration from another field on the same farm or from the farm of a neighbor. Early planting and the use of fertilizer under the corn at planting time often resulted in large enough plants to withstand attack by the time the billbugs emerged. However, spring rains will not always permit early planting.

CHEMICAL CONTROL.—Starting in 1950, an attempt was made at Florence, S. C., to develop a chemical control for billbugs using the new insecticides.

Post-emergence treatments.—All of the tests reported were conduced against *Calendra maidis*, the dominant species in the Florence, S. C., area. Separate tests using both *C. maidis* and *C. callosa* showed these species about equally susceptible to insecticides. The major portion of

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the damage occurs in the spring, which is the only time of the year when the insects are out of the stalks where an insecticide can reach them. Early efforts were directed toward surface applications in an attempt to save the stand. Sprays using wettable powders and emulsions, dusts, granulated formulations, and bait mixtures were applied both as broadcast and row treatments. Different schedules of application were tried, including emergence, first damage, post-cultivation, and weekly applications, in addition to combinations of these. Aldrin, chlordane, DDT, dieldrin, heptachlor, lindane, malathion, and parathion were used at several rates in these tests. Several of these gave indications that they might control the billbugs if properly timed and placed.

A number of billbugs were killed in the tests, but never enough to save the stand of corn. With the billbugs emerging over a 2- to 3-week period, even the best residual insecticide did not give any appreciable kill after about 2 days, and from an economic standpoint, the value of the crop would not permit an expenditure of more than $5.00 an acre toward insecticide applications. In addition, most farmers consider it necessary to cultivate their corn about twice during the period the billbugs are active; thus they cover up any residue that might be present on the surface of the soil. If surface applications were to be practical, one would be needed at the time of emergence and another after each cultivation resulting in excessive costs.

INSECTARY TESTS.—Small plots were found to be unsatisfactory for test work, even when they were 20 rows wide, because of the migratory habits of the insect and the lack of uniformity of the infestations in any given field. In January 1953, insectary tests were begun using soil-surface applications and field-collected billbugs. Specimens used for testing from January through March were collected from corn stubble in infested fields which had not been plowed or disked. In a heavily infested field, one man could collect from 800 to 1,000 billbugs in about 7 hours. Specimens for testing during April, May, and June were collected primarily from clumps of volunteer corn, to which early-emerging billbugs were gathering, and from the corn plants at the edge of fields to which masses of migrating billbugs were swarming. These specimens were kept in 5-gallon screen-covered cans to which several inches of moist soil had been added. The cans were sunk into the ground about 10 inches deep, in a cool, shady location. If given stalks of corn to feed on, about 2,000 beetles can be kept in good condition in one can for about 2 months.

In addition to the insecticides mentioned above, limited tests were made using toxaphene, Dilan, and endrin. The insectary tests showed that aldrin and parathion were the most effective, but parathion did not have enough residual activity, when applied as a surface treatment.

PRE-PLANTING TREATMENTS.—It seemed probable that if a material having residual effectiveness were mixed with the soil better control might result. By this method of application the toxic agent would not be broken down by the sun, and the billbugs would come in direct contact with it while active in the soil during migration or feeding. In the insectary tests made, aldrin at 2 pounds per acre resulted in the highest mortality. These results prompted the following field treatment. A 15-acre field having about 4,000 infested stalks per acre in the fall of 1952 was given a broadcast application of 8 pounds of 25% aldrin mixed with 592 pounds of fertilizer per acre. This fertilizer was immediately disked in just before planting to cotton. Corn was then planted in the adjacent field. Frequent observation of both fields during the period of billbug activity showed a great number of dead billbugs between the rows in the cotton; however, only an occasional plant in the adjacent corn field showed damage. During surveying and collecting in other untreated fields, a dead billbug was very rarely found. Further investigations were therefore made to test the practicability of this method for billbug control.

Table 1.—Per cent mortality of corn billbugs in field cage tests, Florence, S. C., 1954.

Material	Pounds Toxicant per Acre	Formulation: Dust, Per Cent	Dust, Per Cent Kill	Granulated, Per Cent	Granulated, Per Cent Kill
Aldrin	2	2.5	86	5	74
	4	—	—	5	89
Chlordane	2	5	14	5	27
	4	—	—	5	48
Dieldrin	2	1.5	54	—	—
	4	—	—	5	57
Heptachlor	2	2.5	48	—[a]	—
Parathion	0.25	1	6	1	6
Check	—	9% average mortality, all tests			

[a] The granulated heptachlor was later found to be below strength; hence the results are not included.

CAGE TESTS.—In 1954, field tests were made using cages 3 ft.×3 ft.×10 in. with fly screen tops. Each insecticide was applied to the surface of plowed soil and worked into the top 3 to 4 inches with a shovel to simulate disking. A given number of field-collected billbugs (25 or 50) were placed on the soil, and the cage was put over them and sunk into the soil about 5 inches. It was pressed down tight and the soil around the outside was packed to make an effective soil-seal. After a 4 to 6-day period, the cages were removed and the soil screened to recover the insects for mortality counts. Dust and granulated formulations of the more promising materials were tested. The results are summarized in table 1. As in previous tests, the use of aldrin resulted in a higher mortality than with the other materials.

In 1955, further field tests were made using cages 1 ft. ×1 ft.×8 in. rather than the larger ones used previously. This size considerably reduced the time and labor necessary to set up and to take data on the tests, and yet gave enough room for the billbugs to move about. Recovery of the beetles from the soil was better than 99 per cent from these cages. Treatments and counts were made as in 1954, using the materials shown to be most promising in previous tests.

In the first test, wettable powders of aldrin, chlordane, dieldrin, and heptachlor were compared at 2 pounds ac-

Table 2.—Per cent mortality of corn billbugs in field cage tests, Florence S. C., 1955.

Test No.[a]	Aldrin			Chlordane	Dieldrin	Heptachlor			Check
	WP	Granulated	Dust	WP	WP	WP	Granulated	Dust	
1	86.2	—	—	23.7	45.0	68.7	—	—	1.2
2	85.0	50.0	77.5	—	—	72.5	48.7	70.0	1.2
3	80.0	45.0	61.2	—	—	63.7	52.5	46.2	5.0
4	96.2	75.0	98.7	—	—	96.2	91.2	87.5	2.0
Average	86.9	56.6	79.1	23.7	45.0	75.3	64.1	67.9	7.1

[a] All materials applied at 2 pounds actual toxicant per acre. Test No. 1–5-day exposure, No. 2–3 days, No. 3–4 days, No. 4–8 days.

tual toxicant per acre. The results, summarized in table 2, again show that aldrin gave a high mortality, with heptachlor somewhat lower. Dieldrin and chlordane gave inferior kills.

In an attempt to compare the effectiveness of the materials, and of the different formulations of each, three additional replicated, randomized tests were made using wettable powder, granulated and dust formulations of aldrin and heptachlor, each at 2 pounds of actual toxicant per acre. Results are given in table 2. The aldrin again gave a high rate of kill, with the heptachlor nearly as high. The wettable powder formulation was superior to the other forms, and the dust tended to be better than the granulated. Results of test number 4 indicate that aldrin and heptachlor will give a greater degree of control in farm practice, where the residue is protected from the sun, and the exposure of the insects to the toxicant is longer.

Field Tests.—Field tests in 1954 were unsuccessful, because of inadequate methods of locating fields which were suitably infested. However, in the fall of 1954 a survey of over 400 corn fields resulted in the location of 17 farms having heavy infestations. Infestations were determined by crossing each field at several places, pulling a stalk about every 6 to 8 feet, and cutting into the base of the plant to check for teneral adults. A 20% infestation was considered satisfactory for testing control measures. It was concluded that this was the only sure way of determining the presence of an infestation before emergence in the spring.

While these 17 farms had fields with enough billbugs for test purposes, all but three of them were considered unsuitable for test work because of an outbreak of an entomogenous fungus, *Beauveria bassiana*, which had resulted in a 20 to 70% mortality. No dead billbugs were found on the three farms chosen for these tests.

Since aldrin at the rate of 2 pounds actual toxicant per acre was effective in the previous tests, one field on each of two farms was treated as follows: Wettable powder was mixed with fertilizer at the rate of 5 pounds of 40% aldrin to 95 pounds of fertilizer. On March 19, this amounts was broadcast over the plowed field with a fertilizer distributor, giving 2 pounds actual toxicant per acre, which was immediately disked into the soil. Corn was planted in the treated and check fields a week later. On farm number 1, two infested fields had a 200-foot strip of land between them that had been planted to tobacco the previous year. Both infested fields were treated, although only one of them was replanted with corn in 1955. The other field was treated to kill the billbugs before they could migrate to corn. Both fields had a population of nearly 8,000 billbugs per acre going into hibernation the previous fall. A third infested field was located just beyond the first two. It was planted to small grain, while the field beyond it was planted to corn in 1955. These latter two fields served as excellent checks.

On farm number 2, two infested fields were separated by a wide highway. Both fields had a population of over 6,000 billbugs per acre that entered hibernation the previous fall. Each field was planted to corn in 1955; one field served as an untreated check. Billbugs began emerging from hibernation about 25 days after the treatments were made. Counts made during the period of billbug activity are summarized in table 3.

As a further check, six other fields in the area of the treated fields were checked for damage. These had from 10 to 60% loss of stand, and no dead billbugs were found at any time. It is apparent that an excellent degree of control was obtained from the use of 2 pounds of aldrin per acre in these two fields. In interpreting the finding of only hundreds of dead billbugs, when thousands were known to be in the fields, it is believed that the insects which were in contact with the insecticide before they began to emerge from the soil were killed before they reached the surface, while those which were down deeper than the toxicant did not come in contact with the insecticide until they were emerging from hibernation. These reacted the same as those in cage tests. They came out on the surface, lost control of their legs, rolled over on their backs and remained that way until they died. Many such moribund specimens were found in treated fields shortly after emergence started. Furthermore, a moribund or dead billbug was rarely found in an untreated field.

In putting this type of treatment into practice, fields

Table 3.—Summary of billbugs present and plant damage made during the period of insect activity, Florence, S. C., 1955.[a]

Field	Total Number Plants Damaged	Live Billbugs	Dead Billbugs
Farm No. 1			
Treated	4	0	hundreds
Check	62% of stand	hundreds	0
Farm No. 2			
Treated	9	3	hundreds
Check	51% of stand	hundreds	0

[a] Data reported on basis of whole fields in all cases.

suspected of having an infestation of billbugs should be examined in the fall, by the method mentioned earlier, for individuals entering hibernation. Once the land has been plowed, disked, or even pastured intensively, it is too late to make a satisfactory check. If corn is to be planted in a field adjacent to a known infestation, the infested field should be treated, rather than the one being planted to corn, in order to kill many of the beetles before they start to emerge, and the others as they emerge and start to migrate in search of corn.

SUMMARY.—Billbugs annually cause a sizable loss of stand in corn over much of the United States. Work was started at Florence, S. C., in 1950, to evaluate the use of the new insecticides for their control. When various methods of surface applications of insecticides failed to save the stand from billbug damage, experiments were designed to test residual insecticides mixed with the soil just prior to planting. Insectary and field cage tests, in which aldrin and heptachlor were mixed with the soil, resulted in high mortality. Three heavily infested fields were treated with a broadcast, disked-in, pre-planting application of aldrin at 2 pounds of actual toxicant per acre. This treatment resulted in virtual eradication of the billbugs in two of the fields, and excellent control in the third, while near-by check fields lost 51 to 62 per cent of the stand.

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Cattle Louse Control with Treated Rubbing Devices and Their Distribution in South Carolina[1]

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For years it has been realized that there is a need for a more economical way of controlling cattle lice on the small farm as well as on a large-scale operation. Heretofore, the only means of cattle louse control has been by hand dusting on some of the smaller farms and the use of high-pressure sprayers and dipping vats on larger farms. All of these methods are costly, and the method of application is time-consuming and expensive.

Hoffman (1954a) reported that preliminary tests with the self-rubbing device for controlling cattle lice proved to be very satisfactory when using some of the chlorinated hydrocarbon insecticides under Oregon feed-lot conditions. Since this method of controlling cattle lice seemed very practical and economical for use by both small and large cattlemen, tests were set up in South Carolina during the fall and winter of 1954–55 using both commercial and homemade rubbing devices on purebred and grade herds of cattle.

No extensive survey has ever been made on the different species of cattle lice in South Carolina, although the four common species are probably present. A survey for lice was conducted using herds from various parts of the state, numerous livestock auctions and meat packing plants.

MATERIALS AND METHODS.—Three types of rubbing devices were used: (1) The fly-type (Rogoff & Moxon 1952), (2) the louse-type (Hoffman 1954a), (3) the commercial type.[4] The fly-type and louse-type rubbing devices have been described previously and need not be given here.

The commercial made rubbing device used is very similar to the louse-type with one exception—there is only one cable which is extended through a pulley and stretched to the top of a 5-foot post. A 30 to 40 pound weight is attached to the end of the cable. The purpose of the weight is to keep the wrapped cable stretched as tight as possible to permit the cattle to exert more pressure on the device.

The above described devices were placed in cattle lots containing 10 to 70 animals. They were installed in dry places near feeding boxes, salt licks, or watering holes. Hoffman (1954b) states that the best control was obtained when the devices were not placed in bushy or muddy terrain.

The following emulsifiable materials were used in the treatment of rubbing devices for louse control in South Carolina during 1954–55: Carbide and Carbon's BR-5[5], which contains 25% butoxy polypropylene glycol, 25% DDT, and 0.15% lindane; National Vaccine and Serum's Cattle Insecticide[6] which contains 1.2% piperonyl butoxide, 0.35% rotenone, and 0.15% pyrethrins; chlordane; DDT; lindane; lindane-aroclor; malathion, and methoxychlor. The emulsions of these insecticides were mixed in 5-gallon lots with No. 2 fuel oil. They were prepared in the following concentrations: BR-5 5%, Cattle Insecticide 5%, chlordane 2% and 5%, DDT 5%, lindane 0.5%, lindane-aroclor 0.5%, malathion 2% and 5%, and methoxychlor 5%.

Counts were made monthly on all treated and untreated herds. Five animals were selected at random from each herd for each counting, and the number of lice in a 2-inch-square area of the poll, dewlap, topline, top of the

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[4] Manufactured by the National Vaccine and Serum Company, Denver, Colorado.

[5] A product of Carbide and Carbon Chemical Company, New York, N. Y.

[6] A product of National Vaccine and Serum Company, Denver, Colorado.

shoulder, and tailhead of each animal was recorded on data sheets. These data sheets were used to record the number and the species of lice found on the areas checked. At first, the 2-inch-square area was determined by placing a 3- by 5-inch index card with a 2-inch-square area cut in it over the area to be counted. After a considerable number of counts were made using the index cards, they were discarded and the 2-inch-square area was estimated. The lice were found on the hair and skin by parting the hair with a small pair of forceps. Realizing that this method can only give an approximate figure for the louse population of a herd, the following categories, based on five animals per herd, were established to compare the degree of control obtained: (1) 0–25, excellent; (2) 25–50, satisfactory; (3) over 50, unsatisfactory.

EVALUATION OF RESULTS.—During the fall and winter of 1954–55, experiments with three different types of treated rubbing devices and eight different insecticides were conducted in South Carolina (Table 1). Each device was treated with only one insecticide during the entire season. The initial treatment of the device with approximately 1 gallon of insecticide solution was in November. Subsequent treatments followed with approximately 2 quarts of material per device every 2 or 3 weeks.

A 5% solution of DDT was applied to both commercial and louse-type devices. After 4 weeks the cattle using the louse-type device had an average of 1.6 lice per animal. No lice could be found on the animals after the first check period. Eight weeks after being exposed to the commercial device the herd averaged 0.4 louse per animal. No lice were found thereafter.

A 2% malathion solution was applied to both fly- and commercial-type rubbing devices. No lice were found on the treated herds. Lice were reported on the untreated herd in January and by March the population had increased by almost eight times. From data obtained, the 2% malathion solution appeared to give excellent control of cattle lice.

In a herd (herd 6) known to have been heavily infested with lice during past winters, the louse population was allowed to build up from November to January. In January the herd was given access to a commercial-type device treated with 5% malathion. After the device had been used for 4 weeks, few lice could be found on the animals, and after the device had been tested for 8 weeks no lice could be found.

Tests were run during the winter of 1954–55 to determine if 2% malathion applied to Hereford cows by a treated rubbing device had any effect on the cholinesterase activity of the animals. The average delta pH (Hamblin & Marchand 1953) of five different classes of untreated cattle was found to be in the following categories: Hereford bulls, 0.40; Hereford steers, 0.42; Hereford heifers, 0.47; Guernsey cows, 0.40; Holstein cows, 0.41. The average delta pH (Table 2) of the Hereford cows treated with 2% malathion was 0.43. From these data it appears that 2% malathion applied by a treated rubbing device had no effect on the cholinesterase activity of cattle, since the average delta pH of the treated animals falls within the range of the untreated animals.

During December herd 1, using the apparatus treated with 5% methoxychlor, was rated excellent. When the device was not made available during January, the louse population increased from 1.2 to 37.0 for every 10 square inches of animal. The cattle were given access to the rubbing device again and in 2 months the louse population

Table 1.—Cattle lice control on beef and dairy animals with treated self-rubbing devices, 1954–55.

	Herd No.	Number of Animals	Breed[a]	Type Device[b]	Date of First Treatment (1954)	Average Number of Lice per Animal[c]				
						Dec.	Jan.	Feb.	Mar.	Total
Methoxychlor	1	16	Ho	L	11/13	1.2	37.0[d]	5.4	19.2	62.8
Cattle Insecticide 5%	2	20	Ho	L	11/13	0.0	0.0	0.0	0.0	0.0
Lindane 0.5%	3	38	Ho	L	11/13	0.0	0.4	0.0	0.0	0.4
Lindane-aroclor 0.5%	4	53	Ho	L	11/13	0.2	2.2	0.0	0.0	2.4
Check	5	11	Ho			1.4	68.2	54.4	74.8	198.8
Malathion 5%	6	50	G	C	11/13	11.4[e]	21.4[e]	2.0	0.0	34.4[e]
Check	7	12	G			1.2	0.2	8.6	34.8	44.8
Chlordane 5%	8	11	He	L	11/2	0.0	0.0	0.0	0.0	0.0
Chlordane 5%	9	10	He	C	11/2	0.2	0.0	0.0	0.0	0.2
DDT 5%	10	15	A	C	11/11	0.0	0.4	0.0	0.0	0.4
DDT 5%	11	14	A	L	11/11	1.6	0.0	0.0	0.0	1.6
Malathion 2%	12	35	He	F	11/15	0.0	0.0	0.0	0.0	0.0
Malathion 2%	13	40	He	C	11/15	0.0	0.0	0.0	0.0	0.0
Check	14	10	G			0.0	2.6	8.8	17.0	28.4
Cattle Insecticide 5%	15	47	G	L	11/8	0.0	0.2	0.4	0.0	0.6
BR-5, 5%	16	70	G	F	11/8	0.4	0.0	0.0	0.0	0.4
Chlordane 2%	17	50	He	L	11/17	1.0	0.0	0.0	0.0	1.0
Lindane 0.5%	18	44	He	L	12/14		0.0	3.0	0.0	3.0
Check	19	12	G			2.4	65.0	61.0	101.0	229.0

[a] A, angus; G, Grade; He, Hereford; Ho Holstein.
[b] C, commercial; F, fly; L, louse.
[c] Average number of lice on 10 square inches.
[d] Animals removed from rubbing device for 1 month.
[e] Rubbing device not in use.

Table 2.—Cholinesterase activity of Hereford cows treated with 2% malathion in No. 2 fuel oil applied by treated rubbing devices for 4 months, 1954–55.[a] Clemson, S. C.

Cow No.	Age in Years	Weight at Testing	Average Delta pH[b]
122	9	1,500	0.37
445	5	1,100	0.42
220	6	1,200	0.48
147	9	1,200	0.48
273	6	1,200	0.38
Average			0.43

[a] Tested March 29, 1955.
[b] Cholinesterase determinations made by Miss F. Hermenze, nurse-technician, American Cyanamid Company, New York, N. Y.

was still 19.2 per 10 square inches of animal.

A 5% solution of Cattle Insecticide was applied to two louse-type rubbing devices. Excellent control was recorded with both devices. Two rubbing devices treated with 0.5% lindane were installed in the upper and central parts of the state. Excellent control was obtained with both devices. Another apparatus was treated with 0.5% solution of lindane-aroclor. A few immature lice were found during December and January. The overall control results with this apparatus were recorded as excellent.

A fly-type device was treated with a 5% solution of BR-5. During December only 0.4 louse per 10 square inches of animal was recorded. No lice were found during the other check periods. All of the materials used in the control studies demonstrated excellent results. No ill effects were noticed to the animal's skins from the oil used in mixing the insecticides. Rogoff & Moxon (1952) also stated that no ill effects were noticed from the oil which was applied to the treated rubbing devices.

The louse population of the check herds increased steadily throughout the winter. All of the different types of rubbing devices gave excellent results. The authors agree with Hoffman (1954a) as to the use of the louse-type rubber in preference to the fly-type for cattle louse control. Three of the four species of lice attacking cattle in South Carolina are of the blood-sucking type and usually feed with their mouthparts in the skin of the animal, whereas the fourth species is a biting louse and may be found feeding on the surface of the skin. The animal has to exert pressure on the device in order to allow the insecticide to penetrate the hair and reach the lice. This is made possible by the louse-type self rubbing device, because the cables are tighter than those of the fly-type rubber.

The results of these experiments indicate that the best control is obtained when the devices are treated early in the fall and kept treated until early spring. The cost of treating one animal for louse control for 4 months with a treated rubbing device was found to be $0.10, whereas the cost of applying two spray applications was $0.30.

The use of the homemade treated rubbing device for cattle louse control is recommended over spraying for the following reasons: (1) Cost, (2) the animal's natural inclination to rub, (3) the insecticide can be applied with less excitement to the animal, (4) the ease of application, and (5) the rubbing device requires less labor.

Table 3.—Distribution of cattle lice in South Carolina, 1954–55.

Herd Number	Breed	Species			
		H. eurysternus	*L. vituli*	*S. capillatus*	*B. bovis*
		Upper Part of State			
8	Hereford	No lice	*	*	*
9	Hereford	*	*	*	X[a]
10	Angus	*	*	X	*
11	Angus	*	*	X	*
12	Hereford	No lice	*	*	*
13	Hereford	No lice	*	*	*
14	Hereford	*	X	X	*
15	Grade	*	X	*	X
16	Grade	*	X	*	X
17	Hereford	*	*	X	X
18	Hereford	*	X	*	*
19	Grade	*	X	*	X
20[b]	Holstein	X	*	*	*
		Central Part of State			
1	Holstein	*	X	X	X
2	Holstein	No lice	*	*	*
3	Holstein	*	X	*	*
4	Holstein	*	X	X	*
5	Holstein	*	X	X	X
		Lower Part of State			
6	Grade	*	X	X	X
7	Grade	*	X	X	X
21	Grade	*	*	X	X
22	Holstein	No lice	*	*	*
23	Guernsey	*	*	X	*
24	Guernsey	*	*	X	X
25	Guernsey	No lice	*	*	*
26	Guernsey	X	*	X	X
27	Guernsey	X	*	X	*
28	Grade	*	X	X	X
29	Grade	*	X	*	*
30	Hereford	*	*	X	X
31	Grade	No lice	*	*	*
32	Hereford	*	*	X	X
Total		3	13	17	15

[a] X—indicates lice found.
[b] Herd checked 1953–54.

DISTRIBUTION STUDIES.—Results of the cattle louse survey in South Carolina (Table 3) showed that *Solenopotes capillatus* (Enderlin) was the most abundant species, occurring throughout the state in large populations on all breeds of cattle. *Linognathus vituli* (L.) was found in small numbers throughout the state. It occurred more abundantly on dairy and grade cattle. Relatively few *Haematopinus eurysternus* (Fahrenholz) were found. Two Guernsey herds in the lower section of the state and, during the winter of 1953–54, one Holstein herd at Clemson College were found to have this species. *Bovicola bovis* (L.) was found on all breeds of cattle and ranked second only to *S. capillatus*, in abundance in South Carolina.

SUMMARY.—Tests were conducted using both commercial and homemade rubbing devices on purebred and grade herds of cattle for louse control. Three types of devices were used: (1) The fly-type, (2) the louse-type, and (3) the commercial-type.

Eight different insecticides diluted to various concentrations were used to treat the rubbing units, and all of the materials used gave excellent control of cattle lice when the cattle were given access to the rubbing units in the early fall before the louse populations began to increase. Rubbing units treated with a 2% malathion solu-

tion appeared to have no effect on the cholinesterase activity of Hereford cows.

All of the different types of rubbing devices gave excellent results, but homemade-type self-rubbing devices are more economical for use by the small farmers in South Carolina. It was found that the cost of spraying for cattle louse control was three times greater than using the self-rubbing device.

Four species of cattle lice were found: *Solenopotes capillatus*, *Linognathus vituli*, *Haematopinus eurysternus*, and *Bovicola bovis*. All were very abundant with the exception of *H. eurysternus*, and the most abundant was *S. capillatus*.

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Insecticidal Evaluation of Organic Arsenical Compounds[1,2]

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The use of arsenicals as insecticides has steadily declined since the advent of the new organic insecticides. The fact that the once popular lead and calcium arsenates have been largely replaced by more effective compounds, such as DDT, BHC, toxaphene, the organic phosphate compounds, and many others, has presented the manufacturers of arsenicals with a serious economic problem. In an effort to solve this problem these manufacturers are trying to improve the toxic properties of arsenic compounds so that they may be comparative to the present organic insecticides.

The fact that inorganic arsenicals are generally effective as stomach poisons only is one of the main limiting factors in their use. It is highly possible that this restriction might be overcome by incorporating in inorganic arsenic compounds certain organic radicals which could create a contact poisoning effect as well as intensify the toxicity of arsenic as a stomach poison.

The purpose of this study is to evaluate the effectiveness of some of the newer organic arsenical compounds as insecticides. Biological assays were conducted on several species of insects to determine the insecticidal properties of the test compounds as compared with standard insecticides. The data obtained from these assays were further used to determine which organic radicals enhanced or decreased the toxicity of the arsenic containing molecule. The phytotoxic properties of the more promising compounds were also investigated in this study.

Among the earlier publications dealing with organic arsenicals are those of Brinley (1926), Walker & Mills (1927), Ginsburg (1935), Fink & Smith (1936), and Siegler *et al.* (1939). A very extensive investigation was conducted by Yun Fan (1947), who tested organic arsenicals as stomach and contact insecticides on *Tribolium confusum* Duv. Yun Fan states that some organic arsenicals, such as m- chlorophenylarsonic acid and phenyl arsonic acid, compared favorably with the better insecticides and were far superior to acid lead arsenate, sodium fluoride and basic lead arsenate. He concluded that in the phenylarsonic acid series, the meta-substituted compounds generally were more effective than their corresponding ortho- and para isomers.

More recently Riedeburg (1952) reported that arsenomethane As-1,2-disulfide showed a higher degree of toxicity against the confused flour beetle, *T. confusum*, and the German cochroach, *Blattella germanica* (L.), than calcium arsenate, DDT, or rotenone.

MATERIALS AND METHODS.—In insect toxicity studies each of the organic arsenicals was diluted with Attaclay by the impregnation method to a 20% dust. With a few compounds there were no suitable solvents available; in these cases the technical material was simply ground with the Attaclay in a small ball mill grinder. The standard insecticides used in these tests were either factory formulated or prepared in the laboratory by the impregnation method. These standard dusts were used at concentrations generally recommended.

In the phytotoxicity studies the organic arsenicals were diluted in a standard plant nutrient solution to concentrations that ranged from 1:100 to 1:1,000,000. The standards in these tests were run at the same concentrations as the test materials so as to obtain comparable results.

The insects used in this study were the boll weevil, *Anthonomus grandis* Boh: the rice weevil, *Sitophilus oryza* (L.); southern armyworm larvae, *Prodenia eridania* (Cram.); and the cotton leafworm, *Alabama argillacea* (Hbn.).

The method described by Farrar & Reed (1951) was used to obtain newly emerged boll weevils. The adult weevils were held for a period of 3 days before they were used for testing purposes.

A modification of the method described by Hamner (1943) was used to maintain stock cultures of rice weevils. The adult weevils used in the testing procedure were 4 weeks old or less.

Southern armyworm larvae were reared by the method

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suggested by Swingle (1935). Second instar larvae were used in the tests.

Because of the inability to rear the cotton leafworm in the laboratory, it was necessary to make field collections for testing purposes. The larvae used in the test procedure were in the second or third instar.

The prepared dusts were applied to the various materials for exposure to insects by means of a precision vacuum duster originally described by Farrar *et al.* (1948) and later improved by McCallan (1950). The inside dimensions of the bell jar were 8¾ inches in diameter and 14 inches high.

TEST PROCEDURES.—*Boll weevil.*—The methods employed in these tests were developed by Farrar & Reed (1951). Each compound was tested on cotton plants at sample levels of 10, 25, 50, and 100 mg. of 20% dust, and all dosages were replicated at least three times. Two standard insecticides of known toxicity were included in each of these tests. A number of check plants dusted with Attaclay were also included in each test.

A screen cage holding 10 three-day-old adult boll weevils was placed around each plant. These enclosed plants were kept under controlled conditions at 30° C. and 85% relative humidity.

Weevil mortality was recorded 24, 48, and 72 hours after initial exposure to the treated foliage. The corrected mortality was obtained according to the method of Abbott (1925).

Rice weevil.—Kernels of corn were soaked in water for ½ hour after which they were removed and blotted with paper towels to remove the excess water. Three kernels of soaked corn were cut in half and placed in the bottom section of a petri dish 9 cm. in diameter. The inside surface of the top and bottom sections were then dusted with a weighed sample of dust by means of the precision vacuum duster. All the test compounds, including two standard insecticides, were tested at dosages of 25, 50, and 100 mg. per weighed sample. Each dosage was replicated at least two and occasionally three times. Several checks using Attaclay were run with all these experiments.

After the dusts had been applied 20 adult rice weevils were enclosed within each dish and held at a constant temperature of 28° C. and a relative humidity of 80%. The mortality was recorded at 24 and 120 hours, and corrected according to Abbott's formula.

Southern armyworm.—Circular sections of tendergreen leaves (a mustard-spinach type of green, 8 cm. in diameter) were dusted with the various test compounds. The vacuum duster was used to apply the dusts to the leaf sections. This process was done by suspending the leaf section on a wire tripod within the bell jar of the duster. By suspending the leaf section vertically, both sides were uniformly dusted. After the dust had been applied, each leaf section was placed in an individual petri dish with five second instar larvae. These dishes were placed in a constant temperature-humidity room at 28° C. and 80% relative humidity.

All the test compounds were run at sample levels of 25, 50, and 100 mg., and adequate checks were included to determine the normal mortality rates. These results were also subjected to Abbott's formula.

Cotton leafworm.—Only a limited number of the organic arsenicals available were tested for their effectiveness against the cotton leafworm larvae. The procedures used in these tests were the same as the procedures previously described for boll weevil testing. The compounds were run at dosages of 10, 25, 50, and 100 mg. per sample. Two standards were included in each test, plus an adequate number of checks. The results were calculated to the net per cent mortality.

Phytotoxicity.—The phytotoxic properties of arsenomethane As-1,2 disulfide and dichloroarsenomethane As-1,2 disulfide were tested on cotton. Two types of tests were conducted; one on the foliage of cotton and the other on cotton seedlings in a vial test.

The toxicity of the test compound to cotton foliage was determined by dusting young cotton plants with at least two true leaves. Dusts of 1, 5, 10, 25, and 50 per cent concentrations were prepared with pyrophyllite. Two plants were dusted with each dust at the rate of 200 mg. per sample with a vacuum duster. Two plants dusted with 71.2% tricalcium arsenate were used as the standard. Two controls, dusted with pyrophyllite, were also included in the test.

One plant treated with each of the test materials was placed under a glass lantern globe where the humidity was above 90%. The remaining plants were kept under ordinary greenhouse conditions (16 to 24° C.).

In the cotton seedling tests the technical materials were diluted with a plant nutrient solution in ranges of 1 part active material in 1,000,000 to 1 part active material in 100. The roots of the cotton seedlings were severed just above the transition zone and then placed singly in glass vials with 25 ml. of the solution. Each concentration was run either in duplicate or triplicate. The vials containing the plants were placed in a wooden rack in a greenhouse where the temperature ranged from 16° C. at night to 24° C. during the day.

The cotton seedlings were observed periodically and after 1 week the percentages of seedlings injured beyond recovery were recorded.

RESULTS AND DISCUSSION.—The general effectiveness of the compounds tested is given in tables 1, 2, and 3. Arsenomethane As-1,2 disulfide, chlorinated derivatives of arsenomethane As-1,2 disulfide, camphorated arsenomethane As-1,2 disulfide and arsonated toxaphene gave results on the rice weevil, southern armyworm and cotton leafworm comparable to those of 10% DDT, 20% toxaphene and 3% gamma isomer of BHC. Arsenomethane As-1,2 disulfide was reasonably effective against all four species of insects tested. The addition of one chlorine atom to this molecule gave an increase in toxicity, but the addition of two chlorine atoms gave no apparent increase over the toxicity of the parent molecule.

The introduction of two camphene groups into the arsenomethane As-1,2 disulfide molecule produced no apparent increase in toxicity over the parent compound. However, when the concentration of these two compounds was calculated on a mol per 1,000 grams of dust basis the camphene groups definitely enhanced the toxicity of the parent compound. The concentration of arsenomethane As-1,2 disulfide was 0.41 mol per 1,000 grams of dust while camphorated arsenomethane As-1,2 disulfide was 0.27 mol per 1,000 grams of dust. Although these two compounds had the same apparent toxicity,

Table 1.—Comparative effectiveness of several organic arsenical compounds against four species of insects.

CHEMICALS TESTED[a]	NET PER CENT MORTALITY AFTER HOURS INDICATED			
	Boll Weevil 72	Rice Weevil 120	Southern Armyworm 48	Cotton Leafworm 72
Arsenomethane As-1,2 disulfide	42	100	93	100
Chloroarsenomethane As-1,2 disulfide	100	100	100	100
Dichloroarsenomethane As-1,2 disulfide	19	100	100	100
Arsenomethane As-1,2 disulfide (water leached)	31	100	100	100
Disodium salt of arsenomethane As-1,2 disulfide	77	0	0	100
Camphorated arsenomethane As-1,2 disulfide	19	100	100	100
Arsonated camphene[b]	—	43	100	—
Arsonated toxaphene[b]	62	100	100	100
Ethylarsonic acid	77	—	42	64
n-Propyl arsonic acid	36	—	100	60
Phenyl arsonic acid	0	—	45	0
Benzyl arsonic acid	74	—	0	40
p-Hydrophenyl arsonic acid	0	—	0	—
3-Chlorophenyl arsonic acid	88	0	33	—
3-Aminophenyl arsonic acid	—	0	0	—
3-(sulfonic acid) phenyl arsonic acid	40	0	21	—
3-Carboxyphenyl arsonic acid	—	0	0	—
Xylidene arsonic acid	—	9	88	—
3-Nitro 4 hydroxyphenyl arsonic acid	—	0	0	—
4-Carboxyphenyl arsonic acid	—	0	19	—
Xylenol arsonic acid	—	0	73	—
1-Nitro 3-naphthalene arsonic acid	—	99	100	—
m-Nitrophenyl arsonic acid	42	0	0	—
p-Arsanilic acid	0	—	0	10
Cacodylic acid	100	8	76	—
Disodium methyl arsonate	42	—	0	41
Copper methyl arsonate	100	0	60	64
Calcium methyl arsonate	88	0	0	18
Neutral barium phenyl arsonate	42	0	13	0
Neutral calcium phenyl arsonate	8	0	0	0
Antimony arsonate	100	0	50	100
Phenylarsazine chloride	46	0	—	100
Phenylarsine bis (N_1N^1 dimethyl dithiocarbamate)	—	0	10	—
Diphenyl arsine N-dimethyl dithiocarbamate	—	0	13	—
Methyl arsine bis (N_1N^1 phenyl methyl dithiocarbamate)	—	0	0	—
Methyl arsine bis (N_1N^1 pentamethylene dithiocarbamate)	—	0	0	—
Methyl arsine bis (N_1N^1 dimethyl dithiocarbamate)	—	0	0	—
Methyl arsine bis (N_1N^1 morpholine dithiocarbamate)	—	0	13	—
Methyl arsine bis (N_1N^1 tetramethylene dithiocarbamate)	—	0	7	—
20% Tricalcium arsenate	—	46	68	—
56% Tricalcium arsenate	94	—	—	100
BHC, 3% gamma isomer	100	100	—	100
20% toxaphene	51	100	—	100
10% DDT	—	100	100	—

[a] All chemicals tested at a sample level of 100 mg. of a 20% dust.
[b] Received from the American Smelting and Refining Company, South Plainfield, N. J.

Table 2.—Toxicity of arsenomethane As-1, 2 disulfide to cotton foliage.[a]

INSECTICIDE TESTED	EFFECT ON PLANT[b] AFTER			
	48 Hours		120 Hours	
	Humid	Normal	Humid	Normal
1% Dust	1	1	1	1
5% Dust	1	1	1	1
10% Dust	1	1	1	1
25% Dust	2	1	3	1
50% Dust	3	1	4	1
71.2% Tricalcium arsenate	1	1	1	1
Check (pyrophyllite)	1	1	1	1

[a] Applied 200 mg. of dust per plant.
[b] 1, not affected; 2, slightly affected; 3, moderately affected; 4, severely affected.

the actual toxicity of the parent compound was nearly doubled by the introduction of the camphene groups.

When arsenic pentoxide was incorporated into the toxaphene molecule, the apparent toxicity of the toxaphene increased. This increase in toxicity was even more striking when examined on a molar concentration basis, when toxaphene was 0.49 mol per 1,000 grams of dust and arsonated toxaphene was only 0.31 mol per 1,000 grams of dust. Since the exact chemical structure for toxaphene was unknown, these calculations were made using an assumed molecular weight for toxaphene.

SUMMARY.—A total of 39 organic arsenical compounds was evaluated in the laboratory for their insecticidal properties using boll weevils, rice weevils, southern armyworm larvae, and cotton leafworm larvae as test insects. With the exception of the cotton leafworm, all of the insects used were reared within the laboratory. The test compounds were formulated on Attaclay by either impregnation or ground methods, and were applied to the various test media by means of the precision vacuum duster. All the compounds were tested at several dosages with two or three replications of each. Standard insecticides were included in each of the tests.

The most outstanding compounds tested were: arsenomethane As-1,2 disulfide; camphorated arsenomethane As-1,2 disulfide and arsonated toxaphene. Arsonated toxaphene possessed the highest degree of toxicity and was more effective than toxaphene against the boll weevil. Phytotoxicity tests revealed that arsenomethane As-1,2 disulfide and its chlorinated derivative were about as toxic to cotton foliage as calcium arsenate.

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Table 3.—Toxicity of solutions of arsenomethane As-1, 2 disulfide to cotton seedlings.

INSECTICIDES TESTED	PER CENT OF SEEDLINGS INJURED BEYOND RECOVERY				
	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Copper aceto arsenite	100.00	100.00	100.00	38.47	9.10
Tricalcium arsenate	53.85	76.93	9.10	7.70	0.00
Lead arsenate	100.00	100.00	33.33	0.00	0.00
DDT	0.00	0.00	0.00	0.00	0.00
Arsenomethane As-1,2 disulfide	100.00	70.00	12.00	10.00	0.00
Chloroarsenomethane As-1,2 disulfide	100.00	100.00	100.00	0.00	0.00
Check	0.00	0.00	0.00	0.00	0.00

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Experiments on the Control of the Two-Spotted Spider Mite on Strawberries[1]

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The two-spotted spider mite, *Tetranychus telarius* (L.), is the most serious pest of strawberries in southern California. Unless adequately and promptly controlled, it can ruin the crop in a very short time. Experiments on its control with various insecticides were conducted at Stanton, Calif., from 1951 to 1954. Applications were made as needed, usually from April to July. In 1953 they were applied as early as February, but they were not required from August to January.

GENERAL METHODS.—The experiments were conducted on the Lassen variety of strawberry on plots 10 to 50 feet long and 1 to 6 beds wide. The beds were 3 to 3½ feet from center to center, each with 2 rows of plants. The plots were arranged in randomized blocks. Dusts were usually applied with rotary hand dusters equipped with Y nozzles so that both rows of plants in the bed could be treated at once. Concentrated sprays were applied with a high-pressure knapsack mist sprayer. The beds were sprayed once from each side when about 10 gallons was applied per acre, and once from the top when about 5 gallons was applied. The results were checked by counting the live mites on the underside of five leaflets per plot. Except in 1951, applications were repeated when the count showed more than five mites per leaflet.

1951 EXPERIMENT.—In 1951 both dusts and sprays were applied on April 6, May 9, June 14, July 25, and dusts also on April 24. Eight counts were made from April 12 to August 22. As shown in table 1, excellent control was obtained with demeton and good control with Aramite and ovex. Poor control was obtained with schradan, malathion, and parathion. Malathion and parathion gave good control during April and May but were ineffective during July.

The following materials were used at the start of this experiment but were unsatisfactory:

	Dosage per acre
Oil emulsion 1%	8 gal.
5%	10 to 20 gal.
Sulphenone, 10% dust (3 formulations)	33 to 43 lb.
Compound 876 bis(*p*-chlorophenyl)ethynyl carbinol):	
Dust 5%	47 lb.
7%	75 lb.
Spray Em 2 lb. per gal., 1–79	9 gal.
1–159	9 gal.
Em 1.66 lb. per gal., 1–639	267 gal.

The last spray was applied with a conventional power sprayer at pressure of 350 pounds per square inch.

1952 EXPERIMENT.—In 1952 the first insecticide applications were made on April 3. Only 7 of the original 12 materials were used throughout the season. Mite counts were made weekly from April 14 to August 25 and monthly in September and October.

Outstanding results were obtained with the demeton spray in three applications (Table 1). It was significantly better than Metacide, Aramite, TEPP plus Sulphenone, or TEPP dust, which required from 6 to 13 applications. Ovex with five applications was better than 11 to 13 applications of TEPP with or without Sulphenone.

The following materials were dropped after the indicated number of applications because they were ineffective:

Chlorobenzilate 2% dust	3
2 lb., 1–29 and 1–19 sprays	4
Sulfur 25% dust	2
Schradan 4 lb., 1–19 spray	3

Dinitrobutylphenol, 1.1 per cent dust, was dropped after one application because of severe burn.

The 1952 experiment was conducted in highly productive commercial fields; so no check plots were used, and it was necessary to keep the mites under control. Therefore, NPD 4 per cent dust and 1-29 spray, compound 876 10 per cent dust, ovex 10 per cent dust, and demeton 4 per cent dust were substituted for these materials in late applications. The NPD dust was ineffective after

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Table 1.—Control of the two-spotted spider mite with various treatments applied to Lassen strawberry plants at Stanton, Calif., 1951–54.

MATERIALS[a]	POUNDS OF DUST OR GALLONS OF SPRAY PER ACRE-APPLICATION	NUMBER OF APPLICATIONS	NUMBER OF SURVIVING MITES PER LEAFLET
1951 Experiment (4 replications):			(8 counts)
Demeton 3 lb., 1–19 spray	6.9	4	0.4
Aramite, 3% dust	34	5	2.1
Ovex, 10% dust	33	5	3.0
Schradan 4 lb., 1–19 spray	6.4	4	8.0
Malathion, 5% dust	35	5	10.2
Parathion, 2% dust	36	5	11.0
L.S.D. at 5% level			5.5
1952 Experiment (4 replications):			(22 counts)
Demeton 3 lb., 1–19 spray	4.7	3	0.9
Ovex, 10% dust	37	5	3.0
DMC 2 lb., 1–39 spray	9.6	8	4.8
Metacide 4 lb., 1–29 spray	8.2	9	6.1
Aramite, 3% dust	38	7	6.6
TEPP 1.6 lb.+Sulphenone 2 lb., 1–29 spray	8.3	11	8.2
TEPP, 1% dust	39	13	11.0
L.S.D. at 5% level			4.4
1953 Experiment (4 replications):			(23 counts)
Ovex, 10% dust	40	3	2.0
Demeton 2 lb., 1–29 spray	4.5	3	2.2
Compound 876, 10% dust	41	5	3.1
NPD 7.6 lb., 1–19 spray	8.3	7	3.4
Aramite, 3% dust	40	6	3.5
Ovex, 2 lb., 1–39 spray	8.7	6	3.5
Demeton, 2% dust	29	7	4.4
DMC 2 lb., 1–39 spray	8.4	10	4.7
Metacide 4 lb., 1–29 spray	8.5	8	5.2
NPD, 4% dust	40	13	7.2
TEPP 1.6 lb.+Sulphenone 2 lb., 1–29 spray	8.2	16	8.0
TEPP, 1% dust	40	14	8.0
L.S.D. at 5% level			—[b]
1954 Experiment 1 (8 replications):			(13 counts)
Aramite 3% dust	39	2	3.0
Ovex, 10% dust	38	2	3.0
Diazinon 4% dust	40	4	3.0
Holcomb compound 326, 2 lb., 1–4 spray	9.6	3	3.1
Rohm & Haas FW-152 2.8 lb., 1–13 spray	9.6	3	4.3
TEPP 1% dust	48	5	7.5
Chlorobenzilate 4% dust	40	7	8.2
L.S.D. at 5% level			2.6
1954 Experiment 2 (6 replications)			(10 counts)
Demeton 2 lb., 1–19 spray	7	2	4.1
	14	2	1.3
	21	2	2.1
Methyl analog of demeton 5 lb., 1–59 spray	6	2	3.8
	13	2	2.0
	19	2	1.6
Schradan 6½ lb., 1–31½ spray	7	2	2.6
	13	2	2.7
	20	2	1.6
L.S.D. at 5% level	—	—	1.7

[a] Pounds indicate weight of insecticide in 1 gallon of emulsifiable concentrate.
[b] Not significant by the *F* test.

three applications and the spray after two applications, but they were retained in the experiment at higher dosages.

Demeton and ovex were better than Metacide, Aramite, TEPP plus Sulphenone, or TEPP (Table 1). The TEPP was less effective than all the other materials.

1953 EXPERIMENT.—In 1953 new materials were used to replace those found ineffective or damaging in 1952. The first applications were made on February 5 and the last weekly counts on July 13.

Owing to extreme variations between blocks, there was no significant difference between treatments in the average number of mites per leaflet, but the number of applications required to keep the mites at these levels ranged from 3 to 16 and, in general, the most effective materials not only gave the best control of mites but fewer applications were required. One or two applications of an aphidicide for control of the strawberry aphid, *Capitophorus fragaefolii* (Ckll.), would also be required if the grower used ovex, Aramite, DMC, or compound 876 to control the mites, but not with the other materials.

1954 EXPERIMENTS.—Two experiments were conducted in 1954. The first one was in the same field used in 1953, but on a new planting of strawberries. Applications were started on April 27 and counts were made weekly from May 3 to July 26. After 3 weekly applications at 40 pounds per acre, Karathane 3 per cent was found to be ineffective, and it was dropped from the experiment. In its place Rohm & Haas FW-293 5 per cent dust was used for the rest of the season, and it gave promising results.

Of the seven materials used throughout the season, the best results were obtained with Aramite and ovex with two applications, Holcomb compound 326 and Rohm & Haas FW-152 with three applications, and Diazinon with four applications (Table 1). There were no significant differences between these materials, and all were significantly better than TEPP with five applications or Chlorobenzilate with seven. The Rohm & Haas FW-152 spray caused slight marginal leaf burn.

In the second 1954 experiment systemics were used on plots 10 feet long and 1 bed wide. The first applications were made on April 2. Biweekly counts showed less than five mites per leaflet for more than 2 months. A rapid increase occurred between June 14 and 29; so the applications were repeated on July 6.

In the average of the 10 counts there was no significant difference between materials. Three days after the second application demeton and its methyl analog were significantly better than schradan, but 10 days later there was no difference.

The high and medium dosages were better than the low dosages, but there was no significant difference between high and medium dosages.

Residue analyses[2] made 5 days after the April 2 application showed 0.5, 0.6, and 1.7 p.p.m. of demeton from dosages of 6, 11, and 17 ounces of demeton per acre, respectively, and a maximum of 0.2 p.p.m. after 24 days. The maximum residues following the second application (on July 6) were 0.5 p.p.m. after 1 day, 0.2 after 7 days, and less than 0.1 after 14 and 21 days. In another test in which demeton was used at 4, 8, and 12 ounces per acre, analyses showed 0.1, 1.0 and 2.0 p.p.m., respectively, 4 days after the first application (on April 22) and less than 0.1 p.p.m. after 18 and 25 days. A second application (on May 25) showed the following maximum residues: after 1 day 0.2, after 7 days 0.5, after 22 days 0.4, and after 15 and 28 days less than 0.1 p.p.m. It appears that demeton residues are highest following early spring applications.

LEAF BLACKENING WITH ARAMITE.—It has been known for several years that Aramite sprays blacken the upper surface of the leaves and cause a slight browning on the under side. This blackening has never been considered serious, and it was thought that possibly it could be caused by solvents used in the emulsion concentrates rather than by the Aramite. Therefore, in 1953 an experi-

[2] Analyses made by the Pittsburgh Coke & Chemical Co.

ment was conducted on young plants, with 5-plot replicates, in which a 25 per cent emulsifiable concentrate diluted 1–9 and 1–19, and a 90% technical material 1–34 and 1–69 were applied at 7 to 8 gallons per acre, and a 3% Aramite dust at 30 pounds. One application was made on May 1, and counts and examinations of damage were made on May 6, 14, and 22. Leaf blackening was rated as follows: 0, none; 1, slight; 2, medium; and 3, severe.

In mite control all treatments were better than the check, but there were no significant differences between treatments. Significant blackening resulted from all spray materials as compared with the check, and an insignificant amount was found in the dust plots. No blackening had been observed previously from the use of dusts, but extreme hot weather followed this application. *B-1956* was used as the emulsifier in the spray containing technical Aramite. It has been used with several other materials and caused no blackening of leaves. As this spray caused more blackening than those containing the emulsifiable concentrate, it seems safe to conclude that the blackening was due to the Aramite and not to the solvent or solvent emulsifier. The ratings for the different treatments were as follows: Check, none; dust, 0.2; emulsifiable concentrates 1–19, 1.0, and 1–9, 1.2; technical 1–69, 1.6, and 1–34, 2.4.

SUMMARY.—In tests for the control of the two-spotted spider mite, *Tetranychus telarius* on strawberries in southern California from 1951 to 1954, consistently good results were obtained with demeton, ovex and Aramite. Materials inconsistent or comparatively ineffective in most experiments were oil emulsions, schradan, malathion, parathion, Sulphenone, Compound 876, Chlorobenzilate, sulfur, dinitrobutylphenol, DMC, Metacide, TEPP, NPD, and Karathane. Materials showing promise in 1954 but requiring further testing were Diazinon, Holcomb compound 326, the methyl analog of demeton, schradan, and Rohn & Haas FW-152 and FW-293.

Aramite spray concentrates caused some blackening of the upper leaf surfaces, but this is not considered serious. The dinitrobutylphenol caused severe leaf burn, and a spray concentrate of FW-152 caused slight marginal leaf burn.

Damage and Reproduction by the Flour Beetles, *Tribolium confusum* and *T. castaneum*, in Wheat at Three Moisture Contents[1]

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Much work has been done on the biology and behavior of the confused flour beetle, *Tribolium confusum* Duv., and the red flour beetle, *T. castaneum* (Hbst.), in relation to milled grain products but relatively little is known of their relation to whole grains.

Until recently, it has been assumed that the flour beetles found in grain lived either on cracked kernels or on the grain dust resulting from abrasion of grains in handling or on the "flour-like" material resulting from the feeding of other insects. Cotton (1943) stated that the adult flour beetles were able to survive for long periods of time in wheat but thought that only minor damage resulted since the insects were thought to be unable to feed or multiply on whole grain. Good (1936) stated that insects of the genus *Tribolium* were unable to feed upon whole grain because their mandibles were not strong enough to chew through the tough outer coating. Parkin (1944) stated that in different tests "broken grain" was employed because *T. confusum* was unable to feed on whole grain. In U. S. Department of Agriculture Farmers' Bulletin 1260 (1953) the statement was made that "while present in grain shipments, it (red flour beetle) confines its attack in these to grain dust and the surface of broken grain; hence, it is not a primary pest of commercial shipments." After observing the behavior of flour beetles, Birch (1947) reported a feeding experiment in which both species lived and reproduced in a whole grain wheat medium.

The experimental work herein reported had a two-fold purpose: (1) to obtain information regarding the damage to wheat kernels by *Tribolium confusum* and *T. castaneum* and (2) to determine the ability of these insects to reproduce on whole kernels of uncracked wheat.

MATERIAL AND METHODS.—A 6-months' study was made of these insects by infesting lots of wheat with moisture contents of approximately 10%, 12% and 14% at 80° F.

Stock cultures of flour beetles were reared in wide-mouthed pint jars on a food medium of 40% white, patent flour and 60% wheat middlings or shorts.

The moisture content of the wheat was held constant by keeping the cultures in a constant humidity atmosphere maintained by an aqueous solution of sulfuric acid of known specific gravity in battery jars as used by Stevens (1916) in rearing fungi on fruit. Battery jars, 8×15 inches in size and closed with a pane of glass were convenient humidifiers. A trial and error method was used to determine what relative humidity in the jars was necessary to obtain the desired moisture content of the grain. Two battery jars were set up at approximately 50% and 75% relative humidity. Samples of 155 grams of 11.7% moisture wheat in pint jars were placed in each humidifier. Moisture readings of these samples were taken every 2 days by a Steinlite Electric Moisture Tester. In about 10 days, the moisture content of the wheat began to reach an equilibrium with the atmosphere in the humidifier The wheat in the humidifier of 50% relative humidity attained a mois-

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Table 1.—**Relative humidity and specific gravity of sulfuric acid solution required to hold grain at 10.2%, 12.2% and 14.2% moisture contents.**

Relative Humidity Per Cent	Moisture Content of Grain (Per Cent)	Specific Gravity of Acid Solution
60±2	10.2±0.1	1.29
75±2	12.2±0.1	1.23
90±2	14.2±0.1	1.14

ture content of about 9%; at 75% air relative humidity, the wheat attained 12.2 (±0.1)%. Eventually, the correct moisture levels of the grain were reached (Table 1).

Only a small quantity of sulfuric acid solution was required to maintain a constant humidity within a battery jar. The humidifiers were kept in a well insulated stored grain insect rearing room maintained at 80° F. (±1° F.) and a relative humidity of 50 to 60%.

After the wheat had been cleaned and the cracked kernels removed, it was separated into 20-gram samples and placed in the humidifiers. Seventeen 20-gram samples along with one 155-gram sample, which was used as a moisture check, were placed in each humidifier, figure 1. These samples were weighed every 5 days and the moisture content taken every 2 days. In the preparatory stage wheat was added or removed from each sample to maintain the 20-gram weight. At the end of approximately 3 weeks the moisture content of the 20-gram samples was stabilized.

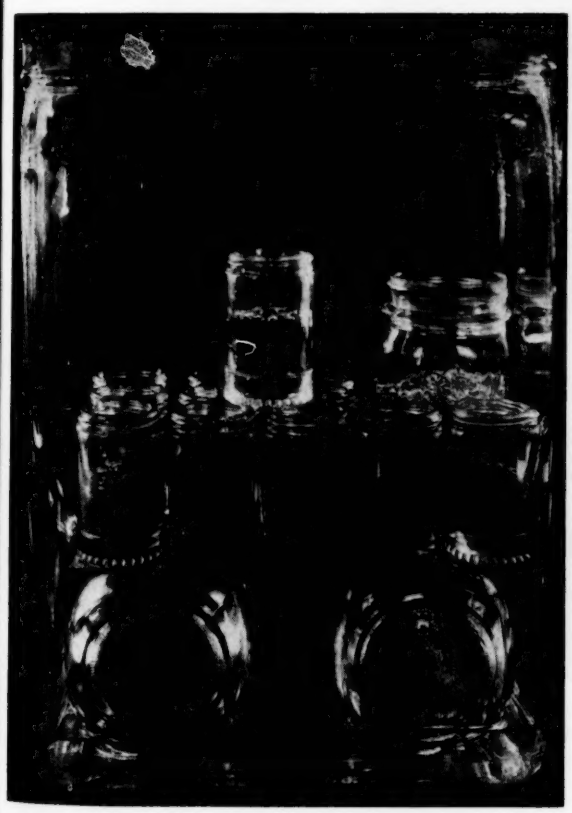

FIG. 1.—Humidity control apparatus containing 12 sample jars and a moisture check jar.

Fifteen of the seventeen samples were infested with 50 adult flour beetles which had emerged 6 to 15 days previously. The two non-infested samples were maintained as weight checks. All were returned to the humidifier along with the two weight control samples. Infestation periods of 2-, 4- and 6-months' duration were selected. Six humidifiers with 15 lots each, were maintained during the complete 6-months' period; three for *T. confusum* and three for *T. castaneum*. Two humidifiers each were maintained for the 10%, 12% and 14% moisture grain. Each wheat sample was replicated five times. Five of the samples from each humidifier were removed and examined at the end of 60 days, five more at the end of 120 days, and the remaining samples at the end of 180 days. The two weight-control samples were weighed and the moisture content of the 155-gram samples was taken at weekly intervals during the 6-months' period.

The weight loss or damage as herein recorded was determined on the basis of the loss in weight of the 20-gram wheat samples with all waste and excrement removed by screening. The difference between the weight loss of the grain less the weight of the waste indicated the feeding loss. At the end of each infestation period, when 30 infested samples were examined, the insects and waste were separated from the wheat by screening. The wheat was then weighed and this weight subtracted from the weight of the control samples. It was found that the wheat did not lose weight immediately after removal from the humidifier or as a result of sifting. This was proved by removing one of the control samples, weighing it, and allowing it to remain in an atmosphere of much lower humidity over a period of 2 hours, then reweighing repeatedly.

The insects were separated from the waste or dust by means of a 100-mesh screen sieve. In this way, the young larvae were easily removed from the dust. The insects were then counted and the various stages determined.

RESULTS.—Results are given in table 2. It was found that the moisture content exerted an important part in the amount of damage to the wheat, the extent of reproduction, and mortality of the insects. For *T. confusum* at the end of 60 days, the average percentage of damage to the 20-gram wheat samples of 10.2% moisture was 1.2% by weight with a total of 20 insects remaining alive. At the end of 120 days, the average percentage of damage was 1.7 with no live insects. The insects did not survive

Table 2.—**The effects of the length of infestation by *Tribolium confusum* and *T. castaneum* in 20-gram samples of whole kernel wheat of three moisture levels as shown by the extent of damage to the wheat and the development of the insect populations.**

Infestation Period (Days)	10.2% Moisture		12.2% Moisture		14.2% Moisture	
	Per Cent Damage	Total Insects	Per Cent Damage	Total Insects	Per Cent Damage	Total Insects
			confusum			
60	1.2	20	2.1	72	3.3	92
120	1.7	0	6.2	84	8.3	134
180	—	—	8.7	102	10.8	134
			castaneum			
60	0.9	0	1.3	15	2.4	55
120	—	—	1.7	18	3.0	56
180	—	—	2.1	24	3.7	53

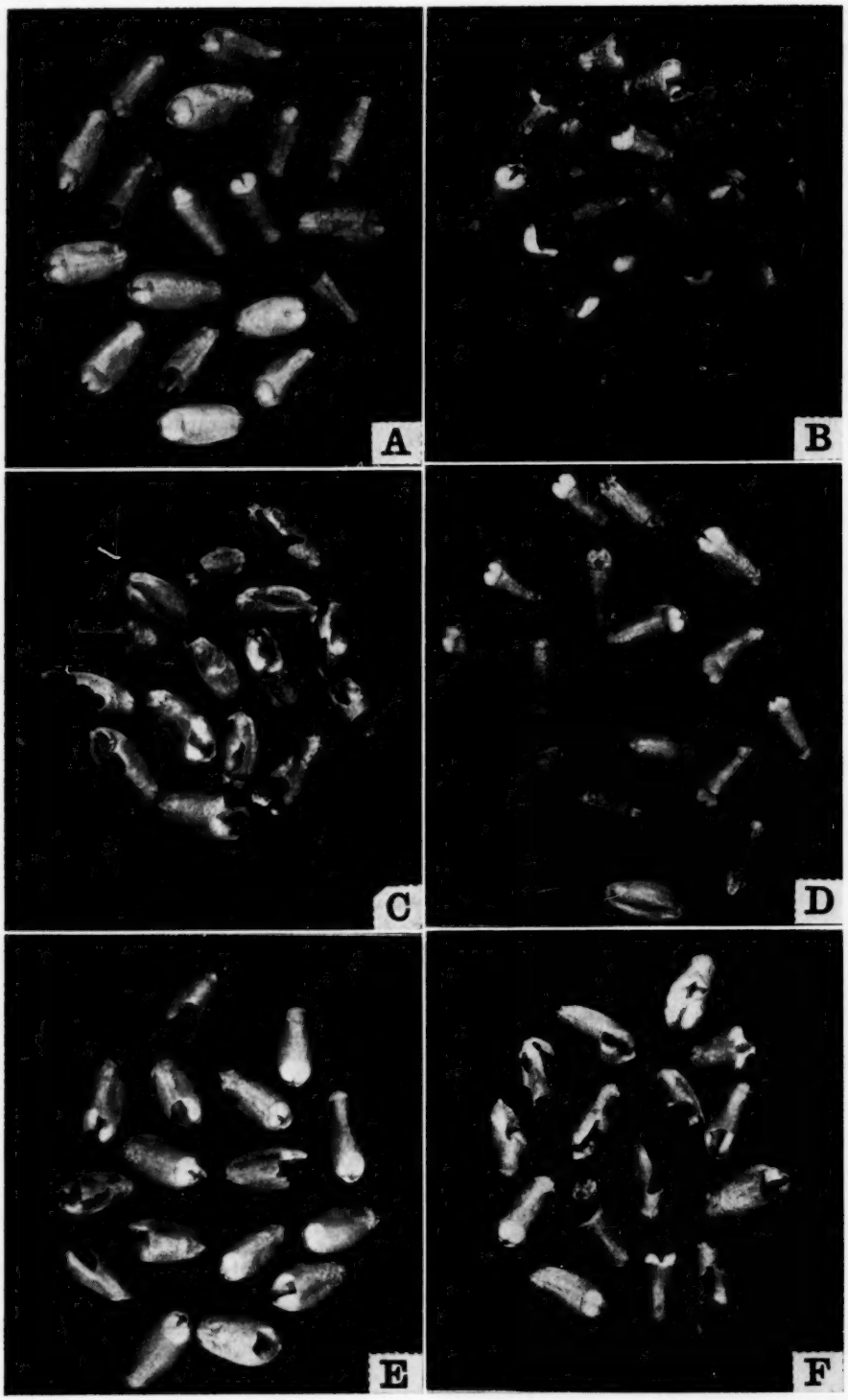

FIG. 2.—Damage by adult *Tribolium* and their progeny to whole kernel wheat of different moisture contents during different infestation periods: A. By *T. confusum* to 10.2% wheat at the end of 120 days. B. By *T. confusum* to 12.2% wheat at the end of 180 days. C. By *T. confusum* to 14.2% wheat at the end of 180 days. D. By *T. castaneum* to 10.2% wheat at the end of 60 days. E. By *T. castaneum* to 12.2% wheat at the end of 180 days. F. By *T. castaneum* to 14.2% wheat at the end of 180 days.

the second 60-day period. For *T. castaneum*, the average percent of damage of the 10.2 wheat was 0.9 with no surviving insects at the end of the first 60-day period.

Tribolium confusum caused more damage to the 14.2% moisture wheat during the second infestation period (60 to 120 days than during the third infestation period (120 to 180 days). It caused 5% damage (8.3−3.3) during the second period compared with 2.5% (10.8−8.3) during the third period. The average total number of insects at the end of 120 days (134) was the same as at 180 days. Holdaway (1932) suggested that the insects reached an equilibrium with the amount of space or food medium in which they lived and many of their immature stages, particularly eggs and pupae, were destroyed. This results in the leveling off of the population and in a lessening of damage to the wheat during the third 60-day infestation period. The beetles did not reach their population equilibrium during the second 60-day period of infestation in 12.2% moisture wheat.

Tribolium castaneum caused slightly less damage to the 14.2% wheat during the second 60-day infestation period than during the third. It maintained about the same number of live insects for 180 days as a result of an increasing rate of adult mortality. The same amount of damage to the 12.2% wheat was done during the second 60-day infestation period as during the third period. *Tribolium castaneum* did not reach its population equilibrium in 180 days. *Tribolium confusum* did greater damage to the grain and had a higher reproductive rate than *T. castaneum*.

In the 12.2% wheat both species showed an increase of larval population, particularly of last instar larvae, in comparison with the 14.2% wheat. Chapman (1918) stated that the life cycle of *T. confusum* reared on different foods under uniform conditions, may vary in length and that the difference is confined largely to the last larval instar. However, in this experiment there was an increase in numbers of the last larval instar when reared on the same food under different moisture conditions. This indicates that the beetles developed and emerged in a shorter time in the 14.2% wheat than they did in the 12.2% wheat.

The natural mortality of the immature stages, as the result of cannabalistic tendencies, under the conditions of this experiment could not be determined. The dead adults were mostly from the original infestation and not of their progeny. The moisture content of the wheat was the most important factor in the mortality of these insects (Table 3). As the moisture was increased, the per cent mortality decreased. There was a much higher mortality rate for *T. castaneum* than for *T. confusum* in the lower moisture content wheat.

Table 3.—Percentage mortality of adult *Tribolium confusum* and *T. castaneum* in 20-gram wheat samples of three moisture levels at the end of the periods indicated.

Per Cent Moisture in Wheat	Per Cent Mortality After			
	confusum		*castaneum*	
	Per Cent	Days	Per Cent	Days
10.2	100	120	100	60
12.2	42	180	85	180
14.2	2	180	40	180

Initial *Tribolium* feeding on undamaged wheat kernels was done on the germ, figure 2, as previously noted by Birch (1947). After eating the germ, the insects fed on the endosperm nearest the germ where its protective covering was weakest. Feeding on the endosperm continued until, in some instances, most of the kernel was consumed. Approximately one-third of the kernels showed feeding damage, regardless of moisture content, though most damage occurred at the higher moisture rates.

SUMMARY.—A 6-months' study was made to determine the amount of feeding damage to whole kernel wheat of 10.2%, 12.2% and 14.2% moisture content at 80° F. by *Tribolium confusum* and *T. castaneum* and to note the amount of progeny development.

Twenty-gram samples of whole kernel wheat of each moisture content were infested with 50 adult insects. Five replications of each infested sample were left for periods of 60, 120, and 180 days. Examinations of these samples were made at the end of each period to determine the weight loss, the number of progeny, and live and dead adults.

The beetles caused the most damage to the wheat with the highest moisture content. There was also greater reproduction in the wheat with the highest moisture level. *Tribolium confusum* caused more damage to the wheat and also reproduced more successfully than *T. castaneum*. At the end of the 120-day period, *T. confusum* had reached a population equilibrium in the wheat with a 14.2% moisture level.

Wheat kernels were first attacked at the germ end. After the germ had been entirely consumed, feeding continued on the endosperm until, in some cases, it was completely consumed leaving only portions of the crease.

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An Unusual Method of Passing the Summer by an Anthomyiid, *Hylemya lupini* (Coq.)[1]

L. C. KUITERT[2] and F. E. GUTHRIE[3]

Bitter blue lupine, *Lupinus angustifolius* L., has been grown extensively as a green manure crop throughout North Central and West Florida. It is the most important winter legume in these areas but, partially due to insect damage, acreages have declined during the past two seasons. One of the principal insect pests is the lupine maggot, *Hylemya lupini* (Coq.), Family, Anthomyiidae. Most of the details of the life history of this insect have been worked out; however, the stage in which the insect passes the summer is not reported in the literature which indicates that most of the anthomyiid flies are active during the summer and overwinter in the pupal stage.

Surveys made during the summer months for larval infestations in wild lupines indicated that the number of wild host plants was small and larval infestations were always negative. Adult flies were first observed in appreciable numbers about the middle of November. The best explanation for the appearance of large number of adults late in the fall seemed to be that they had emerged from pupae which had aestivated.

First evidence of extensive insect damage to lupines in the Southeast was reported by Decker & Bond (1947). They report finding severe infestations of anthomyiid larvae burrowing in the stems of blue lupines at Quincy, Florida. Infestations have been found in all lupine-growing areas from West Florida to Leesburg in Central Florida. Populations have varied considerably in intensity but damage appears to be highest in the area bounded by Quincy, Lake City and Gainesville. The lupine maggot now is the most important insect attacking blue lupines in the Southeast. It is becoming so destructive in some areas, the future of the crop is threatened.

HISTORY.—The species, thought to be native to North America, was originally described as *Phorbia lupini* by Coquillett (1901). Fowler reported finding it in experimental lupine plantings in California, (1902). Chittenden (1908) discussed its importance in the control of loco weeds and briefly described the damage to these plants.

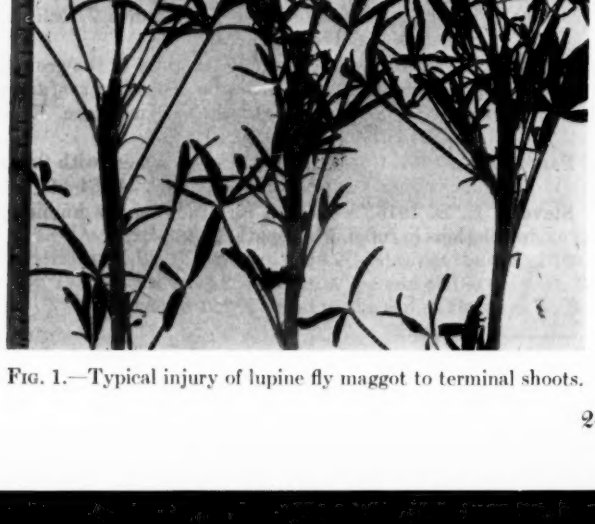

FIG. 1.—Typical injury of lupine fly maggot to terminal shoots.

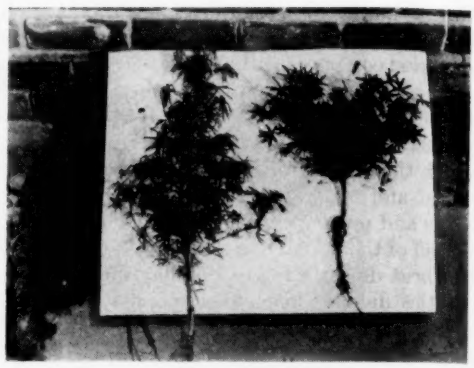

FIG. 2.—Right—Characteristic growth habit of plants heavily infested with lupine maggot. Left—Uninfested plant.

Further investigations on the distribution and damage in Florida were reported by Bond & Decker (1948). Guthrie (1954) gave a preliminary account of the life history and presented data on the duration of the immature stages, longevity of adults, length of pre-oviposition and oviposition periods, and number of eggs laid per female.

NATURE OF INJURY AND DISTRIBUTION.—The seriousness of the injury to lupine plants is dependent on the stage of growth of the plants. Plants infested in the seedling stages are usually killed. Newly hatched larvae usually enter the plant through the terminal bud and burrow downward through the stem. Infested buds wilt, turn brownish and die (Fig. 1.). This feeding is fatal to plants having two or less true leaves. Plants having several true leaves and branches are retarded in growth but not killed. Observations made during 1953 and 1954 revealed that when infested plants averaged 6 to 8 inches in height, uninfested plants of the same age were 18 inches (Fig. 2.).

OVER-SUMMERING STAGE.—During 1953 studies were initiated at Gainesville and Quincy to determine in what stage the insect passed the summer. The rather sudden appearance of appreciable numbers of adult flies in late fall indicated it was highly probable that this anthomyiid fly passed the summer in the pupal stage. In so far as is known, there are no published reports indicating that anthomyiids over-summer in the pupal stage.

The following procedures were followed at Gainesville in an attempt to determine the stage in which the insect passed the summer.

Procedure 1.—A number of well developed larvae found feeding in the lupines were collected and placed in rearing cages. These almost-mature larvae were collected May 11, 1953, well toward the end of the lupine growing season.

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Provisions were made for the larvae to pupate under fairly normal conditions. Observations were made frequently until most of the larvae had pupated. The cage was examined for emergence of adults at approximately 2-week intervals during the summer and fall. Three dead adult flies were found on November 23, 1953.

Procedure 2.—Twenty-eight pupae were collected by sifting soil taken from fields of lupines known to have been severely damaged by the maggots. Pupae were most frequently found approximately 2 to 3 inches below the surface and within 6 to 10 inches of the base of the plant. On June 16, 1953 these pupae were placed in soil to depths approximating that at which they were found. This soil was then placed in a cage out-of-doors. Water was added to the soil at frequent intervals. Examinations were made periodically for the appearance of adult flies. Seven flies were collected from this cage. The first adults emerged on November 8 and the last on November 20.

Procedure 3.—In early May, 1953, a large cage (6 ft × 6 ft. × 6 ft.) was placed over heavily infested lupines growing in the field. Soil, adjacent to but outside the cage, was examined monthly during the summer until 10 pupae were found. By finding pupae present in the soil near the cage it was assumed that pupae could be found in the caged area and it was therefore not necessary to disturb the soil inside the cage.

On November 25, 1953, the first adult flies were found inside the cage. A total of 19 flies were collected. Four additional collections were made at weekly intervals. Adults taken numbered 40, 91, 68, and 14 for these collections. Several spiders were found inside the cage when the last three counts were made. It is not known whether the spiders had any significant effect in reducing the fly population. It is assumed that the flies had to come from pupae present in the soil at the time the cage was placed in position since there was no other means by which this number of flies could have entered the cage. During this same period, numerous adults were observed in the area.

The following procedures were used at Quincy: One hundred mature larvae were collected from field grown plants on April 15, 1953. The larvae were placed in petri dishes having the bottom lined with dry filter paper. All larvae pupated within a few days. After pupation was completed, the petri dishes were maintained in the laboratory and observed weekly for emergence of adults. No attempt was made to alter the humidity in the petri dishes. The first adult fly was observed on October 4. Actual emergence may have occurred 3 to 4 days earlier. The last date of emergence was November 20. A total of 10 adult flies emerged.

In the 1954 tests, made at Quincy, an attempt was made to shorten or break the aestivation period of the pupae by various means. Pupae were collected on May 5, 1954, by screening soil from a lupine field known to have been heavily infested. One hundred pupae were collected in this manner. These were divided into 4 lots of 25 each. Each lot was placed in a petri dish having the bottom lined with filter paper and maintained under these conditions: (1) in laboratory, no moisture added; (2) in laboratory, moisture added to filter paper periodically, paper moist at all times; (3) in laboratory, no moisture added—petri dishes placed in refrigerator for 1 week each month. This treatment was repeated four times during the holding period; and (4) buried about 2 inches deep in soil of foundation planting near laboratory.

Table 1.—Dates of emergence of *Hylemya lupini* (Coq.) collected at Quincy, Florida, May 5, 1954.

No. Collected	Subjected to	No. Emerged	Dates of Emergence
25	Dry	5	October 25 to November 29
25	Moisture added	4	November 10, 12, 16, 17
25	Alternated Cold-warm	5	October 25; November 11–20
25	Outdoors	1	November 21

A total of 15 adult flies emerged. Dates and periods of emergence are given in table 1. No explanation can be given for the low percentage of emergence in the lot of pupae buried in the soil adjacent to the laboratory. Results indicate 16 to 20% emergence in the other lots. Exposure of pupae to alternate hot and cold conditions had no effect on the length of the pupal period.

Guthrie reported that adult flies will emerge in about 2 weeks from pupae which are collected in the field during the months of December to March and placed in petri dishes at temperatures of 70° F. But when these pupae are subjected to temperatures below 60° F., the pupal period requires a month or longer.

POSSIBILITIES OF CULTURAL CONTROL.—Early in the spring of 1953, it was learned that one of two fields in which observations were being made was to be plowed while the other was to be saved for seed. Corn would be planted in the former field and no crop was planned following seed harvest in the latter. It was inferred that both fields would be planted to lupines in the fall. These fields were less than one-half mile apart. Thus it seemed apparent that information could be obtained on the value of reducing incidence of the lupine maggot by plowing under infested lupines and growing a crop requiring frequent cultivation.

During late March, surveys were made to determine the population and damage in the two fields. Individual plants having over 50 maggots and more than 100 damaged terminals were found in both fields. Plants were approximately the same size in the two fields; however, individual plants varied considerably. For this reason, the insect counts were considered an inadequate basis on which to evaluate the populations.

No suitable means of determining the populations were known and only estimates could be made. In general, the populations were considered about uniform in the two fields; perhaps slightly larger in the field which was to be saved for seed. In addition, in the latter field, breeding continued into early May. In October-November, 1953, both fields were again plowed and planted to lupine. Seeding of the two fields was completed within a period of 5 days. On December 3, 1953, a survey was made to determine the prevalence of damage. Plants in both fields were 5 to 10 inches in height at the time of the survey. One hundred seedlings were examined at three places in each field. Examinations were made at 10-yard intervals from the south margin of each field, and the results are given in table 2.

The data indicate that plowing under infested lupines may be an effective means of reducing infestations by this insect. It is assumed that frequent cultivation contributed to this reduction in incidence. Considerable loss

Table 2.—Number of Seedling Lupines Found Infested with *Hylemya lupini* (Coq.) in Cultivated and Non-cultivated fields.

Yards from Field Margin	Cultivated		Not Cultivated	
	Infested	Not Infested	Infested	Not Infested
1	8	92	93	7
11	11	89	90	10
21	12	88	92	8
Totals	31	269	275	25
Per Cent	10.3	89.7	91.6	8.4

of seedling plants could be avoided by early planting. Observations indicate that stands of seedling lupines are considerably reduced by the maggots under conditions of heavy infestation. Planting lupines well in advance of the emergence period of the adults would allow the plants to develop considerably and thus avoid attack in the very vulnerable seedling stage.

Conclusions.—The data indicate conclusively that *Hylemya lupini* (Coq.) aestivates in the pupal stage in the soil of infested fields. Adult flies emerge in approximately 2 weeks from pupae collected in the field during the months of December to early April. Pupae collected during May and June remain in the pupal stage for periods of 6 months or more before adults emerge. The authors do not know of any published reports indicating that any of the other anthomyiid flies aestivate in this stage.

Some evidence indicated that an appreciable reduction in damage can be obtained through cultural means. Early planting will aid in reducing the number of seedlings destroyed by the maggots. Crops requiring frequent cultivation should follow lupines, since tillage apparently destroys large numbers of pupae.

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Effects of Schradan on Tetranychid Cotton Mites[1]

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Mites belonging to the family Tetranychidae are common pests of cotton in the cotton growing areas of the world which occasionally cause serious damage to the crop. Attempts to control these mites by spraying with lime sulphur solution have been made (Parker 1913). DDT has, however, been found not only ineffective against these mites, as reported by Helson (1945) and Potter & Perkins (1946), but in some cases increases in population have been reported by Dean (1946), Chandler (1945), Klostermeyer & Rasmussen (1953). Lime sulphur solution, which is in general use, has some limitations. Firstly, it is readily washed away by rain; secondly, the infestation of mites being on the lower surface of the leaves careful spray of the lower surface is essential for an effective control; thirdly, the residual effect remains for a very short period. The authors, therefore, carried out some experiments with the systemic insecticide, schradan, to determine its effectiveness in the control of these mites.

Methods.—The schradan used contained 66 per cent of the anhydride. This was obtained through the courtesy of M/S Pest Control Ltd., Cambridge, U. K. Schradan contains not only the insecticide called octamethylpyrophosphoramide, $(Me_2N)_4P_2O_3$, but also its higher homologue $(Me_2N)_5P_3O_5$ (Lickerish 1951).

Plants grown in earthen pots were sprayed with 0.3% and 0.5% of schradan solution in water. The experiment was replicated six times. Three leaves in each plant were selected at random at three different heights for observation. The population of the mites on the leaves at the top, middle and base was noted 24 hours before treatment and 24 and 72 hours after treatment. For population counting the "Imprint Technique" adopted by Montgomery (1948) on counting mite populations on fruit trees was adopted. A wooden roller was slowly passed over a folded blotting paper, in the fold of which the infested leaf was kept. This process left impressions of the dying mites on the paper, which were counted. Total area of the leaf surface was found by tracing the leaf outline on a graph and the population per unit area was thus determined. Percentage mortality due to the insecticide was found by the decrease in population per square inch and the net percentage mortality due to inseciticde was calculated by applying Abbot's formula. During the experiment the temperature ranged between $82.2 \pm 13.5°$ F. and the average relative humidity was 40%.

Results and Discussion.—The percentage mortality of mites in 24 hours due to 0.3% concentration was 94.4, 92.7 and 75.9 in the leaves at the top, middle and base, respectively. In case of 0.5% concentration it was 95.8, 93.9 and 92.7 for the top, middle and base, respectively, in 24 hours. But in case of control the percentage mortality was only 40.7 in the leaves at the top and nil in case of middle and basal leaves (Table 1). An increase in the population of mites by 37.8% and 39.8% on the middle and basal leaves was also noted (probably due to hatching of eggs during the period of experimentation). The percent-

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[2] Thanks are due to Dr. S. N. Banerjee, Head of the Section of Entomology for providing facilities for carrying out these experiments. The Commonwealth Institute of Entomology, London, kindly identified the mites.

Table 1.—Percentage mortality of cotton mites before and after treatment with schradan.[a]

Per Cent Concentration Applied	Time of Count[b]	Position of the Leaves on Plants: Top Mites per Sq. In.	Top Mortality	Middle Mites per Sq. In.	Middle Mortality	Base Mites per Sq. In.	Base Mortality
0.3	A	27.3	—	22.7	—	8.8	—
	B	1.5	94.4	1.7	92.7	2.1	75.9
	C	1.0	96.2	1.1	95.2	3.5	71.2
0.5	A	13.5	—	25.0	—	23.6	—
	B	0.6	95.8	1.5	93.9	1.7	92.7
	C	0.3	99.0	0.8	96.7	0.8	96.5
Control	A	28.2	—	25.5	—	9.8	—
	B	16.7	40.7	35.2	—[c]	13.7	—[d]
	C	23.1	18.2	22.6	11.4	12.6	—[e]

[a] Average of six replications.
[b] A, 24 hours before treatment; B and C, 24 and 72 hours, respectively, after treatment.
[c] Increase of population by 37.8%.
[d] Increase of population by 39.8%.
[e] Increase of population by 27.9%.

age of mortality of mites in 72 hours due to 0.3% concentration was 96.2, 95.2 and 71.2 in the leaves at the top, middle and base, respectively. In the case of 0.5% concentration after 72 hours the percentage mortality was 99.0, 96.7 and 96.5 in the leaves at the top, middle and base. In the case of control however, the percentage mortality after 72 hours was only 18.2, 11.4 and nil in the top, middle and basal leaves, respectively. There was also an increase in the population by 27.9% in the basal leaves in control plants during the same period. Thus it is quite evident that 0.3% and 0.5% concentrations of schradan are both very effective against the mites. The data were analyzed statistically and net percentage mortality was calculated according to Abbott's formula (Table 2).

The net percentage mortality in 24 hours in the leaves at top, middle and base in case of 0.3% concentration of schradan is 90.5, 94.7 and 82.7, respectively. In case of 0.5% concentration the net percentage mortality is 92.9, 95.6 and 96.2 in the same leaves during the same period. Thus 0.5% concentration apparently shows a better effect than 0.3% after 24 hours. The net percentage mortality in 72 hours in case of 0.3% concentration is 95.4, 94.6 and 77.5 in leaves at the top, middle and base, respectively, whereas in case of 0.5% concentration it is 98.8, 96.9 and 97.2. Thus the 0.5% concentration was also more effective after 72 hours than the 0.3% concentration. Our observations are also in accordance with the observations of Ivy *et al.* (1950) who stated "Octamethyl pyrophosphoramide appeared to be highly specific for aphids and mites."

Table 2.—Net percentage mortality of cotton mites shown in Table 1.[a]

Interval After Spraying (Hours)	Position of Leaf	Concentration of Schradan: 0.3 Per Cent	0.5 Per Cent
24	Top	90.5	92.9
	Middle	94.7	95.6
	Base	82.7	96.2
72	Top	95.4	98.8
	Middle	94.6	96.9
	Base	77.5	97.2

[a] Calculated according to Abbott's formula.

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Some Effects of Two Hurricanes Upon Populations of Potato-Infesting Aphids in Northeastern Maine[1]

W. A. Shands,[2] G. W. Simpson,[3] and H. E. Wave[2]

In 1954 advantage was taken of an opportunity to determine some effects of two hurricanes in northeastern Maine upon populations of four species of potato-infesting aphids—the buckthorn aphid (*Aphis abbreviata* Patch), the green peach aphid (*Myzus persicae* (Sulz.)), the potato aphid (*Macrosiphum solanifolii* (Ashm.)), and the foxglove aphid (*Myzus solani* (Kltb.)). Observations were made on Irish potatoes on Aroostook Farm, near Presque Isle and on swamp rose (*Rosa palustris* Marsh.) and Canada plum (*Prunus nigra* Ait.) at widely separated locations in central and southern Aroostook County. Hurricane "Carol" passed over northeastern Maine from late afternoon of August 31 until early morning of September 1, and hurricane "Edna" struck on September 11.

Readings at a weather station about 2 miles from Aroostook Farm showed that wind velocities during the first hurricane averaged 23.0 to 28.8 m.p.h., with gusts up to 49.5 m.p.h. while during the second one they averaged 26.5 to 36.8 m.p.h., with gusts up to 51.8 m.p.h. Because of its higher elevation the velocities at Aroostook Farm may have been still higher. Rainfall at the farm amounted to 0.39 inch during the first hurricane. During the second one it was approximately 6 inches over northeastern Maine. Foliage of potatoes and other plants was damaged on both occasions. The wind twisted and agitated the foliage so severely that some potato leaves were broken, and the undersides of many leaves were exposed to the driving rain. Many potato leaves were held so long in this twisted state that they did not return to a normal position for several days. During the first hurricane the raindrops were smaller than usual but rain fell lightly during most of the storm. Heavy rain fell throughout the second hurricane.

Aphid Populations on Potatoes.—Based on the three-leaf method of counting aphids (Shands & Simpson 1953), table 1 shows by species the effect of the first hurricane upon populations of wingless aphids on Green Mountain potatoes in a field well removed from wind obstructions. No insecticide had been applied to the plants. The variation between plots in size of aphid population before the hurricane was due to cultural treatments in the experiments for which the plots were grown.

The overall population of wingless aphids was reduced 48.9 per cent by hurricane "Carol" (Table 1). By species, the reduction ranged from 68 per cent for the green peach aphid to 1 per cent for the foxglove aphid. For the more abundant species the reduction was 52 per cent for the buckthorn aphid and 32 per cent for the potato aphid. Observations on September 1, following this hurricane, disclosed many dead aphids stuck to the soil on the ridges beneath the potato plants, and others on the soil still alive but injured. Apparently the wind and driving rain forced the aphids from the foliage.

Aphid Populations on Swamp Rose.—Based on the whole-leaf method of counting aphids (Shands & Simpson 1954), table 2 shows for 1953 and 1954 the numbers of wingless potato aphids and the population increases on swamp rose in central and southern Aroostook at comparable times after the beginning of the fall migration. The percentage for 1953 represents the increase to be expected normally when the fall migration of this aphid is in progress. The average increase at 11 stations in 1953 was 244 per cent, whereas at the comparable time in 1954 it amounted to only 73 per cent. The much-reduced increase in 1954 was due largely to the effects of hurricane "Edna."

Aphid Populations on Canada Plum.—Fall migration of the green peach aphid to its primary host, Canada plum, began about September 9 in the southern part of

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Table 1.—Effect of 1954 hurricane "Carol" upon populations of wingless aphids infesting Green Mountain potatoes in a field near Presque Isle, Maine.

Plot No.	Aphids on 3 Leaves per Plant, 25 Plants per Plot								Per Cent of Change (All Species)
	Buckthorn		Green peach		Potato		Foxglove		
	Before	After	Before	After	Before	After	Before	After	
1	183	223	218	55	151	191	28	13	−16.9
2	315	198	2	8	45	46	27	20	−30.1
3	905	410	146	18	169	71	2	1	−59.1
4	628	432	0	4	174	98	0	4	−32.9
5	1351	501	37	20	249	73	37	1	−64.5
6	2445	543	6	0	153	62	4	0	−76.8
7	228	475	19	48	30	32	55	35	+77.7
8	2513	1355	47	2	59	57	162	238	−40.6
9	74	12	3	0	57	72	5	18	−26.6
10	210	119	0	0	38	69	14	0	−31.7
Per cent of change	−51.8		−67.6		−32.3		−1.2		−48.9

Table 2.—Effect of 1954 hurricane "Edna" on populations of wingless potato aphids on swamp rose in central and southern Aroostook County, Maine.

Location	Number per 100 Leaves		Per Cent Change	
	1–3 Days Before Hurricane	At Comparable Time in 1953	3–6 Days After Hurricane	After Comparable Interval in 1953
1	2	62	−100	− 85
2	37	17	+103	+682
3	32	77	− 19	+104
4	9	75	− 11	+754
5	13	33	+346	+218
6	11	10	+355	+480
7	3	33	0	+ 52
8	11	17	+291	+100
9	20	9	− 80	0
10	30	78	+ 23	+232
11	24	37	+ 17	+135
All locations	17	30	+ 73	+244

Aroostook County but not until considerably later in the central part. As with the potato aphid on swamp rose, counts indicated that the second hurricane prevented its normal fall build-up on Canada plum. Periodic counts on this plant at 18 locations throughout the fall disclosed only traces of this aphid at any time, and specimens were found at only six locations. The hurricanes, together with the smaller than usual summer populations on potatoes and other secondary hosts, resulted in very few overwintering eggs being deposited on Canada plum. Most likely, eggs of this aphid were deposited in only a small percentage of the thickets. A survey conducted in late June of 1955, when evidence of green peach aphid breeding is normally most abundant, revealed only 2 out of 24 thickets infested with the aphid, with a single specimen taken from each of these thickets. Late-July counts in 1955 disclosed only trace infestations in commercial potato fields in central and southern Aroostook County. The 1955 observations thus corroborate the indicated effects of the two hurricanes in 1954.

DISCUSSION.—Based on our experience, the reduction in aphid population from the first hurricane starting on August 31, was enough to cut aphid feeding damage to a point below the threshold for reduction in yield of tubers. Thus, the hurricane relieved the plants of enough aphids for the feeding damage from those remaining to be below this point. Since the aphid population on the potatoes was in its seasonal decline, it did not again exceed the threshold.

The hurricane of September 11 materially reduced the potentialities for fall populations of the potato and green peach aphids on their primary hosts, with consequent reduction in the number of overwintering eggs and spring populations on these plants.

The factor chiefly responsible for the far-reaching effects of the hurricanes was the time of their occurrence, which resulted in triple-action damage to the aphids. They came when fall migrant forms of the potato and green peach aphids were beginning to mature on and move from secondary to primary hosts, thereby killing many of those that were still on their secondary hosts, those migrating to the primary hosts, and those already on the primary hosts, including progeny recently deposited.

The effects of the hurricanes were apparently much less pronounced upon infestations of the buckthorn aphid. Fall populations of this aphid on its primary host, alder buckthorn (*Rhamnus alnifolia* L'Her.), were large and egg deposition had started before September 1. Furthermore, this low-growing plant occurs in sheltered environments where wind speed is reduced.

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The Mating Range of the Honey Bee[1]

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The honey bee, *Apis mellifera* L., mates in flight at some indeterminate distance from the hive. Thus mating is difficult, if not impossible, to observe. In the past, the lack of a suitable distinguishable genetic characteristic prevented the positive identification by offspring sampling of matings between marked queens and drones.

Sladen (1920) concluded that no cross-matings occurred between virgin queens from one island and drones either from 8 miles away on another island or 11 miles away on the mainland. Klatt (1928) obtained a high percentage of successful matings when virgin queens were located 3.1 and 9.4 miles out on a droneless peninsula 4.4 to 5 miles across water from the mainland. Klatt (1929) obtained similar results at the outer station the following year. No matings were obtained, however, when virgin queens were located 20 miles out on another droneless peninsula, 9.4 to 10.6 miles across water from the mainland. Klatt (1931) obtained matings of virgin queens from colonies separated from drone colonies by 6.3 miles along a peninsula 11.2 miles across water from the mainland. The same author (1932) described the work of Evenius, who was unable to obtain matings between virgin queens from a droneless island and drones from colonies 7.2 miles distant on the mainland. Klatt, repeating an earlier experiment, obtained a high percentage of successful matings of virgins from colonies separated from drones by 5.6 miles along a peninsula, 11.2 miles across water from the mainland. Roberts (1944) demonstrated that a group of queens mated with drones, the nearest of which were from colonies 1.7 miles distant.

The simple recessive (Mackensen 1951) mutation, cordovan, which changes black body pigment to brown or cordovan, was used in this study as a genetic marker to determine mating range. This mutation is particularly suitable as a marker, because (1) there is no evidence to indicate that it is in any way harmful, (2) cordovan bees are easily recognized, (3) populations of any size can be utilized, (4) the mutation is recessive and therefore can be used in the same area for two seasons, and (5) the mutation is not widely disseminated and thus can be used with reasonable certainty that the experimental cordovans will be the only ones in an experimental area. Care must be taken, however, to assure that no swarms are lost.

In an attempt to establish the mating range, the worker progeny of cordovan queens mated at locations varying in distance and direction from a cordovan drone source were examined to determine whether cross-mating of these mutant bees had occurred.

Methods.—Fifty homozygous cordovan queens instrumentally inseminated with sperm from cordovan drones were introduced in a test apiary during September 1953. Although some of these queens were superseded in 1954, the daughters were cordovan, and since drones are produced parthenogentically, only cordovan drones would be expected in this apiary. Examination of the colonies on numerous occasions throughout the season indicated this to be the case.

A search of the area around the test yard revealed 30 colonies of wild-type bees 3 miles north-northwest and 20 colonies 3.5 miles south. These colonies were examined twice during the season to make certain that they contained only wild-type drones. On both examinations only non-mutant bees were observed. Although only 2 apiaries of wild-type bees were found in the immediate area surrounding the test apiary, it is possible that other colonies were present.

Hybrid cordovan virgin queens were reared using a standard double graft technique. These virgins were unrelated to the test-apiary drones, and thus would be expected to carry different lethal alleles (Mackensen 1951). No brood viability problems should result therefore from matings of these virgins and drones.

Twelve nuclei were located permanently in the test apiary. Forty-eight droneless nuclei were located in groups at varying distances and directions from the yard. The distances separating the nuclei, from which the virgins were mated, from the drone yard were measured to the nearest tenth of a mile on an aerial photograph map.

Throughout the season queens were mated during seven periods. The nuclei were moved farther and farther afield from the test apiary as cordovan matings were obtained. Shaded situations along roadside fence rows were sought as locations. Drone brood in the nuclei was destroyed; nevertheless, a few adult drones emerged but these were killed during the frequent nuclei examinations. This was necessary because, since the queens were cordovan, the drones would be cordovan and would bias the results if they were allowed to mate.

The nuclei were fully utilized by mating one group of queens from them while, at the same time, rearing the brood from the preceding group. This procedure had one disadvantage in that queens from two groups were mated before the brood from the first of these emerged. Therefore data on whether or not cordovan matings were being obtained were delayed two mating periods.

Twenty-one days after a group of laying queens were removed from the nuclei their newly emerged worker offspring were examined for cordovan and wild-type. It is worthy of note that no difficulty was experienced in differentiating between mutant and wild-type bees.

Results.—Table 1 presents a summary of the offspring types produced by 336 queens mated during the seven periods from nuclei at the 25 different locations. Some cordovan offspring were produced at all of the sites but one 4.7 miles west, where only four queens were mated. Only 8 of the 76 queens located in the mutant drone apiary produced all cordovan offspring; two produced all wild-type offspring. Three of the 13 queens mated from nuclei located in the 20-colony wild-type yard 3.5 miles

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Table 1.—Numbers of queens from 25 locations[a] that produced the various offspring types.

Miles and Direction Between Cordovan Queens and Drones	Cordovan	Cordovan and Wild-Type	Wild Type
0.0 —	8	66	2
0.1 N	3	10	—
0.5 S	—	8	—
0.5 NW	2	8	—
0.7 E	1	9	—
0.9 W	—	6	—
1.0 S	1	12	1
1.1 N	—	9	2
1.3 S	—	10	1
1.9 S	—	17	4
2.0 N	—	8	—
2.5 S	—	5	7
3.0 S	—	4	2
3.0 E	—	6	—
3.0 N	—	4	3
3.5 S	—	3	10
3.5 W	—	3	1
4.0 S	—	4	9
4.0 N	—	11	15
4.7 W	—	—	4
4.8 NW	—	4	17
5.5 N	—	5	4
5.7 N	—	8	1
5.8 NW	—	2	6
6.0 W	—	1	9
Totals	15	223	98

[a] Mated during seven periods from April to September 1954. Location 0.0 was within the cordovan drone yard; 3.5 S. was within a 20-colony non-cordovan yard; none of the other queen locations were within the immediate vicinity of colonies.

south produced some cordovan offspring; 4 of the 13 queens mated from nuclei 4.0 miles south of the test yard (0.5 mile beyond the 20-colony wild-type yard) produced some cordovan offspring. Furthermore, 15 of the 26 queens, mated from nuclei located 5.5 to 5.8 miles from the test yard, produced some cordovan offspring. Only one of the 10 queens mated from nuclei located 6 miles from the test apiary produced some cordovan offspring.

DISCUSSION.—Queens and drones cross-mate when their colonies are separated by distances up to 6 miles. These results agree with those of Klatt (1928, 1931, 1932). Probably they cross-mate when separated by greater distances; however, this has not been tested, as yet, using mutation marked stock. Unfortunately, this investigation gives no indication of the respective distances flown by drones or queens during mating.

There is no evidence to suggest that the cordovan mutant has a detrimental effect on mating since a relatively large percentage of the test queens mated to cordovan drones at distances up to 6 miles.

Although the test apiary was stocked with 50 colonies of cordovan drones, only a small percentage of the cordovan virgins located in or close to this yard mated exclusively with these drones. The probable explanation of this is that, during mating flights, both queens and drones fly several miles from their colonies. Since there were wild-type drones in the area and since queens mate with several drones (Taber 1955), only a small percentage of them would be expected to mate only with cordovans.

The cordovan virgins located as far as 0.5 mile from the test drone yard apparently mated as purely as the virgins located in the yard. If both queens and drones fly several miles during mating flights, this would have a tendency to reduce the difference in mating purity between queens located at a drone source and queens located near it.

Under the conditions of this experiment, the direction of the mating nuclei from cordovan drone source seems to have had little effect on the amount of cross-mating of the mutant bees. This is surprising in that the size of the wild-type drone populations was different in the various directions. The effect may be so minimized by the flight distances of queens and drones, however, that it is difficult to measure with small numbers of queens in an area where the wild-type drone population could not be controlled or adequately surveyed.

The data presented strongly indicate that, with the exception of isolated island-mated hybrids, the honey bee stock produced and shipped by commerical breeders is extremely variable genetically. Furthermore, uniform honey bee breeding stock cannot be developed and maintained without instrumental insemination. In order to propagate selected stock for commerical use, it will be necessary to provide rigid control of all of the stock for more than 6 miles around a natural mating apiary.

SUMMARY.—The mating range of the honey bee was studied, during the summer of 1954, by the use of stock with the recessive mutation, cordovan, which causes brown body coloration in place of black.

Cordovan virgin queens were mated from droneless nuclei located in and at various distances and directions from a mutant cordovan drone source. The worker offspring from these queens were identified cordovan or wild-type to determine with what type, or types, of drones the queens had mated. Where cordovan workers were produced, they were proof that the queens had mated with drones from the mutant drone apiary.

Offspring type data were obtained from 336 mutant queens mated from 25 different locations, at distances up to 6 miles from the mutant drone source. Cordovan and wild-type offspring were produced at all the locations except one, which was 4.7 miles west. Only four queens were mated from this site.

The direction of the mating nuclei from the cordovan drone source appears not to have been a factor in the amount of cross-mating between these mutant bees. Cordovan virgins located as far as 0.5 mile from the cordovan drone apiary, mated as purely (to cordovan drones) as virgins located in the apiary.

The results of this experiment strongly indicate that commercial breeding stations are not sufficiently isolated to produce and maintain genetically pure stock.

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The Effect of Placement of Insecticidally Treated Transplanting Water on the Control of Wireworms in Tobacco[1]

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The standard recommendation in the flue-cured tobacco belt for protecting newly-set tobacco plants from wireworm attack is the addition of an insecticide (heptachlor, lindane, chlordane, or aldrin) to the transplanting water. This method has generally been satisfactory when the treated water has been applied in hand setters at rates of 200 or more gallons per acre. Where the treated water has been applied in the most commonly used types of mechanical setters, however, poor plant protection has often been observed.

The primary purpose of tests herein reported was to obtain more conclusive data on the effectiveness of hand and mechanical setters in applying treated transplanting water to newly-set tobacco for wireworm control. Secondary objectives were to investigate the possibility of improving wireworm control with mechanical setters by the addition of an accessory water placement valve and to compare the effectiveness of a wettable powder and an emulsifiable concentrate when used in transplanting water.

REVIEW OF LITERATURE.—In California, Morgan & Lyons (1950) effectively controlled wireworms in newly-set tomatoes by adding lindane to the transplanting water. Their tests were conducted with a mechanical setter which dispersed a continuous flow of treated water at the surface and at planting depth at the approximate rate of 600 gallons per acre. Begg (1952), in Canada, reported effective control of wireworms in newly-set tobacco by the use of insecticidally treated transplanting water applied by hand and with mechanical setters, although he did not compare the effectiveness of the two methods of application. Allen *et al.* (1954) reported results of 4 years' experimentation in South Carolina with various insecticides and formulations in transplanting water applied to tobacco transplants with hand setters. None of the literature examined reported research on the comparative effectiveness of different methods of treated transplanting water placement in controlling wireworms.

Allen *et al.* stated, "In general, the emulsifiable concentrates tested either failed to control the wireworms or adversely affected the treated plants when used at concentrations of more than 2 fluid ounces per 50 gallons of water." They reported the identity of the solvents of only a few of the formulations tested but indicated the need for research on the role of solvents and emulsifiers in producing phytotoxicity.

LOCATIONS OF TESTS AND SPECIES OF WIREWORMS.—Wireworm counts were made in a number of fields in eastern North Carolina during the first few months of 1955. One-half acre sections of the three most heavily infested fields were selected as plot land. These three fields respectively were located near the following towns: (1) Whiteville, (2) Lumberton, and (3) Wilson.

At the Whiteville location the soil was somewhat darker, heavier, and more poorly drained than the average tobacco land. It had been planted in corn in 1954. The soils at the other two locations were sandy loams which had produced satisfactory tobacco in 1954. At Wilson, however, the soil appeared to have somewhat higher clay content and was better drained than the other soils.

Wireworms found during the pre-planting counts and while scoring plants in the spring were later identified. At Whiteville all of the specimens examined were species of *Conoderus* but none could positively be identified as *C. vespertinus* (F.), the tobacco wireworm. All of the wireworms collected at Lumberton and Wilson were *C. vespertinus* (F.).

EXPERIMENTAL PROCEDURE.—Three similar experiments were conducted, one at each of the above locations. The experimental design was a randomized block with eight treatments (including a check) replicated four times at each location. Each plot consisted of a single row of tobacco 40 or 50 plants long. The treatments are listed in table 1. In each experiment transplanting water was applied with (1) a hand transplanter (Fig. 1) which placed the water and plant in a cone-shaped depression in the soil, (2) a tractor-drawn mechanical setter (Fig. 2) which opened a furrow into which water and plants were placed simultaneously, and (3) the same setter as (2) with an accessory water placement valve (Fig. 2b) in operation. These setters will be referred to as hand, normal mechanical, and modified mechanical setters respectively. Where the modified setter was used, a part of the water was deposited in the furrow conventionally and a part was delivered to the top of the soil around the plant stem (Fig. 3) through the accessory valve.

Each method of water placement was tested with a wettable powder and emulsifiable concentrate containing 25% of heptachlor applied at 4 ounces of concentrate to 50 gallons of water as is normally recommended for tobacco. The emulsifiable concentrate contained a mixture of ethyl acetate and isopropyl alcohol as the solvent. In most plots 200 gallons of transplanting water per acre was used. This rate was somewhat higher in certain of the

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Table 1.—Tobacco wireworm control with treated transplanting water as affected by water placement and type of formulation.

TREATMENT NO.	TYPE OF SETTER	FORMULATION[a]	GALLONS OF TRANSPLANTING WATER PER ACRE			AVERAGE PER CENT[b] OF MODERATE TO HEAVILY DAMAGED PLANTS		
			Experiment			Expt. 1 (200 Plants per Treatment)	Expt. 2 (160 Plants per Treatment)	Expt. 3 (200 Plants per Treatment)
			1	2	3			
1	Hand	E.C.	200	200	200	2.02	6.45	2.88
2	Hand	W.P.	200	200	200	18.20	17.70	6.92
3	Normal mechanical	E.C.	200	200	225	9.85	37.70	47.90
4	Normal mechanical	W.P.	200	200	225	17.90	41.15	54.40
5	Modified mechanical	E.C.	200[c]	270[d]	225[c]	6.42	17.87	32.00
6	Modified mechanical	W.P.	200[c]	270[d]	225[c]	12.32	27.95	45.28
7	Normal mechanical	E.C.	400	400	450	13.22	28.08	40.95
8	Check	—	200	200	200	33.65	49.25	25.12
		L.S.D. at 5% level				9.75	10.75	9.89
		at 1% level				13.27	14.66	13.46
		Coefficient of variation				47%	26%	21%

[a] Heptachlor 25% emulsifiable concentrate (E.C.) and 25% wettable powder (W.P.) at 4 ounces of formulation per 50 gals.
[b] Transformed to angles for statistical treatment.
[c] 128 gals. to bottom of furrow and 72 gals. to soil surface around base of plant.
[d] 154 gals. to bottom of furrow and 116 gals. to soil surface around base of plant.
[e] 125 gals. to bottom of furrow and 99 gals. to soil surface around base of plant.

mechanically set plots, as shown in table 1. Note that treatment 7 (Table 1) consisted of applying twice the rate of transplanting water with the normal mechanical setter as in the majority of treatments. The mechanical setters were calibrated so that the desired amount of water was released around each plant. Since the hand setter could not be accurately calibrated, the hand-operated water release valve was removed, holes were punched in the bottom of the water reservoir, and an exact amount of water was poured through the setter and around each plant.

Results were evaluated on the basis of wireworm injury to the transplants. Seven days after transplanting, alternate plants in each plot were carefully pulled and taken to the laboratory for examination. After washing, each plant was placed in one of the following categories according to the extent of wireworm feeding: (1) clean, (2) slight (hole less than ½ diameter of stem), (3) moderate (bored at least ½ diameter of stem) and (4) heavy (bored twice or bored and tunneled longitudinally). The rest of the plants in each plot were subjected to similar examination 14 days after transplanting.

RESULTS AND DISCUSSION.—There was little difference in the percentage of plants attacked by wireworms at 1

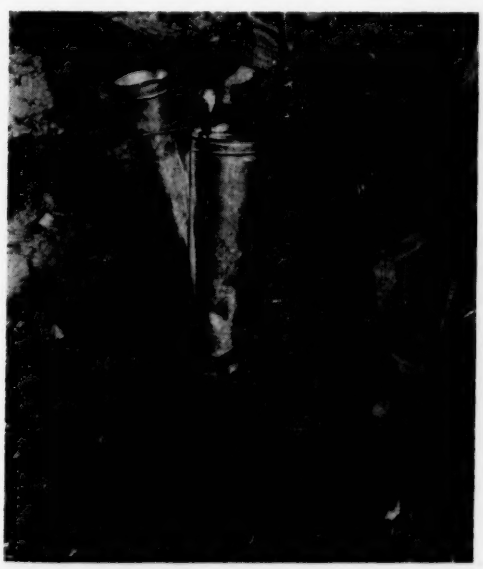

FIG. 1.—A hand transplanter held above a cone-shaped depression made by forcing the transplanter shovels into the soil.

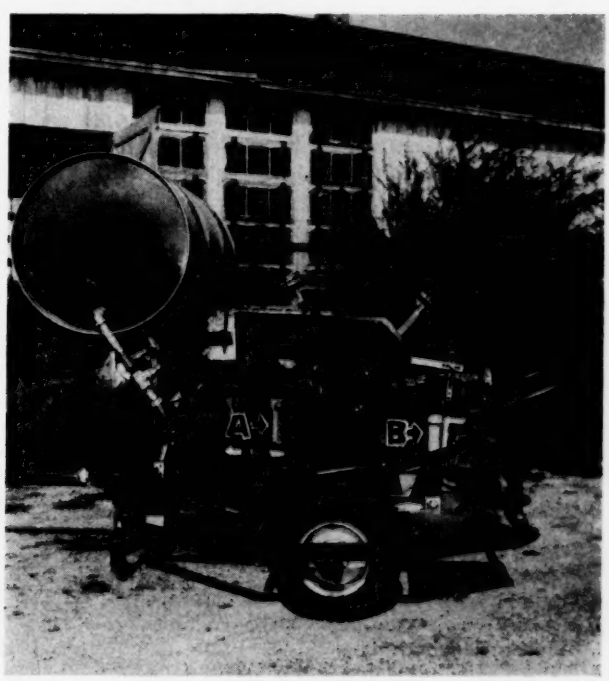

FIG. 2.—A tractor-drawn mechanical setter with (a) a conventional and (b) an accessary water-placement valve.

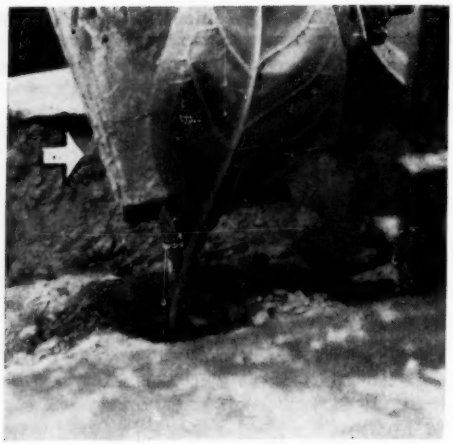

FIG. 3.—The nozzle (see arrow) of the accessory water-placement valve and the newly-set plant. Note dampened soil surrounding plant stem.

and 2 weeks after transplanting. Note that the average of these two counts is given in table 1, in which the results of each of the three tests are summarized.

In Experiment 1 all treatments gave satisfactory control. The fact that no particular method of application was significantly better than another may have been influenced by the extremely high moisture content of the soil at setting. Where the hand setter was used, the emulsifiable concentrate gave significantly (at 1% level) better control than the wettable powder. Differences between the two formulations when applied by the mechanical setters were not significant at the 5% level but were also in favor of the emulsifiable concentrate.

In Experiment 2, the hand setter was distinctly the most effective implement for applying treated transplanting water. The modified mechanical setter, though less effective than the hand setter, gave significantly better results than the normal mechanical setter. Wireworm damage in plots set with the latter setter was almost as heavy as in the untreated plots. The difference between the 200 (Treatment 3) and 400 (Treatment 7) gallon rates of application with the normal mechanical setter was in favor of the higher rate but was not quite significant at the 5% level. The emulsifiable concentrate again gave consistently better control than the wettable powder; however, the difference between the two formulations was significant at the 5% level only where the hand setter was used.

In Experiment 3 weather and soil conditions had a pronounced effect on the results. High temperatures prevailed during and for several days after transplanting, and no rain fell during the experimental period. The soil, having a fairly high clay content, had a tendency to cake when wet to the surface. This caking was only noticeable in plots set with hand setters where the roots and stems of the majority of plants were observed to be encased in a hard core of soil. The wireworms were apparently deterred or repelled by this hard core of soil because the untreated plots set with the hand setter were not as heavily damaged as the treated plots set with the mechanical setter. This physical factor of caking undoubtedly contributed heavily to the highly significant differences between treatments applied with hand setters and those applied with mechanical setters. Otherwise, the data on methods of application were consistent with results obtained in Experiment 2. The emulsifiable concentrate gave significantly better control than the wettable powder where the modified transplanter was used. The differences in control obtained with the two formulations when applied with the hand and normal mechanical setters, though not significant, were in favor of the emulsifiable concentrate.

The observed differences in wireworm control attributable to different types of setters seem obviously due to differences in treated water placement. When a hand setter is used, the transplant and a quantity of treated water are placed simultaneously in a cone-shaped depression in the soil. When the hand setter is removed, some soil falls or is pushed by the operator into the depression around the stem of the transplant. Since in most tobacco soils, the water usually puddles temporarily in the depression, it wets the soil about the stem of the transplant to the soil surface, thus providing an insecticidally treated barrier around the entire underground portion of the transplant. Wireworms cannot, in such cases, reach the transplant without first passing through treated soil. It has been observed that water does not puddle in the depression made by the hand setter in cloddy fields. In such fields, which are particularly prevalent in the upper piedmont district of North Carolina, it is questionable if good wireworm control can be obtained with treated transplanting water even when applied in hand setters.

The mechanical setter utilized operates on the same principle as most of the setters currently used in the flue-cured belt. It opens a continuous furrow into which plants are dropped at regular intervals. A valve delivers a quantity of water from a tank to the bottom of the furrow as each plant is dropped. Shoes or rollers then force soil into the furrow about the stems of the transplants, and thus the transplanting operation is complete. Observation has revealed that the setting water runs down the furrow opened for the transplant and seldom wets the upper portion of the soil around the plant stem. Thus, wireworms can move to the plant stem through untreated soil. It was thought that by doubling the usual rate of application (Treatment 7) the treated water might wet the soil to the surface and thereby give better control. The increased rate seemed to give somewhat better control in Experiments 2 and 3, but the improvement was not significant at the 5% level.

The accessory valve on the modified mechanical setter was an attempt to obtain the same water placement as with a hand setter. That this attempt was only partially successful can be ascertained by the data on control already presented. Though the timing of this accessory valve was considered excellent, the water it released missed a small percentage of the plants. Perhaps the most significant feature of the water placement by the accessory valve, however, was that it resulted in a rather erratic distribution as compared to that obtained with the hand setter where the treated water wet the soil concentrically from the plant stem. Modification of the accessory valve, improved timing of water placement, and increased volume of water directed to the upper layer of

soil around each plant are possibilities which may profitably be studied for improving control with mechanical setters.

When applied similarly the emulsifiable concentrate gave consistently better results than the wettable powder in each experiment, although some of the differences were not significant. One possible explanation for this difference in control is that the wettable powder was apparently more difficult to keep in suspension than the emulsifiable concentrate. Another contributing factor may have been that the wettable powder particles in suspension were filtered out to some extent as the transplanting water passed through the soil.

No phytotoxic effects attributable to either formulation could be determined. It seems possible that the phytotoxic effect of certain emulsifiable concentrates tested by Allen *et al.* (1954), referring particularly to retardation of plant growth or stunting, may have been due to solvents now known to cause plant injury. Some difference in results may be attributed to location. It seems worthy of mention that even when untreated water is splashed on tobacco transplants during bright hot days, the leaves often sun scald. This was quite noticeable in the hand set plots, particularly at the Wilson location. As the hand setter was picked up and moved to the next hill, a few drops of water nearly always dropped on the foliage of the plant just set. In the modified transplanter, a few drops of water from the accessory valve often splashed on the lower leaves of the transplants. Where water was placed only in the furrow with the conventional water placement valve of the mechanical setter, no such damage occurred. Sun scald at transplanting is only a temporary injury, and it is very doubtful if any commercial leaves are damaged; however, some delay in growth may occur.

SUMMARY.—In three similar wireworm control experiments transplanting water treated with heptachlor, both as a wettable powder and an emulsifiable concentrate (containing a mixture of ethyl acetate and isopropyl alcohol as the solvent) was applied to tobacco at transplanting. Three different water-placement devices were used: (1) a hand setter, (2) a tractor-drawn mechanical setter with a conventional water-placement mechanism, and (3) the latter setter with an accessory water placement valve.

Although the treatments did not perform in precisely the same manner at each location, the following conclusions seem justified:

Setting water treatments applied with hand setters gave excellent protection from wireworm attack.

Similar treatments applied with a tractor-drawn mechanical setter, equipped with a conventional water-placement mechanism, did not give satisfactory wireworm control in two of the three experiments. Control obtained with 400 gallons of treated water per acre was not significantly better than that with 200 gallons.

Treatments applied with the mechanical setter equipped with an accessory water placement valve gave better control than when the latter valve was not used. However, control was not as good as with the hand setter.

The emulsifiable concentrate gave consistently better wireworm control than the wettable powder. No plant injury attributable to either formulation could be detected although both treated and untreated water when splashed on foliage at times produced some sun scald.

Observations on growers' farms, and during these experiments, indicate that good control with treated transplanting water can be obtained if a core of soil completely surrounding the stem and roots of the transplant is wet, as produced when hand transplanters are utilized. At the present time, however, the majority of flue-cured tobacco is set with transplanters which do not give the type of water placement necessary for good wireworm control.

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ERRATUM

On page 21 of Vol. 49, No. 1, the figures should be interchanged. As printed, the legend for figure 1 applies to figure 2 and vice versa.

SCIENTIFIC NOTES

Control of the Juniper Scale[1]

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The juniper scale, *Diaspis carueli* Targ., has become an important pest of junipers in the northeastern states, and attempts to control it have led to conflicting reports concerning the success or failure of some insecticides. Preliminary tests at the Bartlett Tree Research Laboratories, Stamford, Connecticut, showed that the common recommendations failed to give even a fair degree of control.

The use of lime sulfur has been recommended from several sources. For instance, Houser (1940) used it and controlled the scale "perfectly." However, a spray of dry lime sulfur at the rate of 20 pounds per 100 gallons of water on April 4, 1955, at Stamford, Connecticut was ineffective. Rough counts before and after the application indicated no kill whatsoever. Use of this material by field men of the F. A. Bartlett Tree Expert Company likewise resulted in failure to control the pest.

Becker (1952) suggested the use of superior type oil in a dormant spray. Unfortunately, this has caused some injury and temporary loss of color, and is generally not favored on juniper. Similarly the dinitros are avoided because of possible damage to the plants themselves or to nearby foliage.

Parathion or malathion applied in mid-May before egg laying begins is the preferred summer treatment according to Matthysse (1954). The former is objectionable because of health hazards, and the latter proved to be ineffective on two trees sprayed on May 3, 1955, at Stamford, Connecticut. In addition, malathion was found to have no appreciable effect on second instar nymphs in a preliminary test at Stamford, Connecticut. The use of DDT for the crawlers is objectionable because of the subsequent buildup of mite populations.

Schread (1955) found malathion effective against crawlers when applied on July 2, 1954, with 1 pound of toxicant per 100 gallons of water giving good though not complete control. These tests were carried out on parts of only two trees.

Timing of a crawler spray is made difficult by inconsistent reports regarding the juniper scale's life history. Schuder (1951) mentions two generations per year, whereas observations in the present study indicate there is only one. Schread (1955) found only one generation in Connecticut during 1954. Emergence of crawlers began on June 3, 1955, at the Bartlett Tree Research Laboratories and extended through July 1, with most of them having left the mother scale by June 20. This coincides closely with the findings of Schread (1955).

A large ornamental planting of junipers (including varieties of the following species: *J. communis* L., *J. horizontalis* Moench, *J. virginiana* L., *J. sabina* L., *J. scopulorum* Sarg., and *J. squamata* Buch.-Ham) at the Bartlett Tree Research Laboratories, Stamford, Connecticut, was selected for test purposes. All were naturally infested, with the greatest number of scales occurring on *J. squamata* var. Meyeri Rehd. From each plot, five healthy tips were taken on June 12 before insecticides were applied and the number of crawler scales on a 3-inch section of each tip was counted under a binocular microscope. On July 13, after all sprays were applied, a second series of five healthy tips was collected from each treatment and the number of living nymphs on a 3-inch section of each tip was likewise determined under a binocular microscope.

Two materials, malathion and lindane, were used. In one series of plots the malathion wettable powder was used alone at the rate of 4 pounds of 25% material per 100 gallons of water, and in the other it was used at the same rate plus lindane wettable powder at the rate of 1 pound of 25% material per 100 gallons of water. All materials were applied with a 50-gallon hydraulic sprayer operating at a pressure of 225 pounds per square inch. Each plant was sprayed thoroughly from the ground with a single nozzle gun. Two check plants were left untreated. Those plants receiving only one application were treated June 20, (at the peak of crawler emergence), those with two applications June 20 and July 5, and those with four June 10, 20, 28 and July 5. Nineteen plants received four applications of malathion, two others were sprayed twice, and two were sprayed only once. Eleven plants were sprayed four times with the mixture of malathion and lindane, while three received two applications and seven were treated only once.

The results (Table 1) show that the scale was reduced 81% by a single application of malathion wettable powder at the rate of 4 pounds of 25% material per 100 gallons of water. The same material applied twice, once at the peak of crawler emergence and again 2 weeks later, gave 100% control. The addition of lindane to malathion did not appreciably increase control.

Some injury was caused by the application of four sprays. This was greatest where malathion was combined with lindane, and the degree of injury appeared to be associated to some extent with the variety involved. Plumose specimens suffered the most conspicuous damage which consisted of a temporary loss of color. All injured plants were growing vigorously and exhibiting natural color by September.

Table 1.—Control of the juniper scale with summer sprays, Stamford, Connecticut, 1955.

Materials Used per 100 Gallons of Water	Number of Applications[a]	Number of Plants	Average Number of Scales per Inch of Tip[b]		Per Cent[c] Control
			Before Treatment	After Treatment	
Malathion W. P. (25%) 4 lbs.	4	19	9	0	100
	2	2	3	0	100
	1	2	6	2	81
Malathion W. P. (25%) 4 lbs. plus Lindane W. P. (25%) 1 lb.	4	11	7	0	100
	2	3	5	0	100
	1	7	4	1	85
Untreated	—	2	4	7	—

[a] Time of sprays: 1 application—June 20; 2 applications—June 20 and July 5; 4 applications—June 10, 20, 27, and July 5.
[b] Based on counts of five 3-inch sections of healthy tips.
[c] Determined by Abbott's formula.

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Automatic Device for the Collection of Aquatic Specimens[1]

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Surveying mosquito breeding areas to determine the presence and incidence of aquatic mosquito stages has always been recognized as a fundamental effort in organized preventive medicine programs which are concerned with the mosquito as a disease vector. The method of choice to accomplish an aquatic mosquito survey has been, from the beginning, the inspection of suspected water surfaces by means of a series of dipped samples usually made with a white enamelled dipper, although a great variety of similar-sized receptacles have been used. In order to complete the sampling effort the mosquito stages present in the dipped sample must be removed and transported to the laboratory for accurate counting and identification. The first part of this latter operation is subject to a certain amount of error on the part of the individual who must have both the ability to recognize and the patience to remove all aquatic mosquito stages present with the aid of an eye-dropper or spoon. When large aggregates of aquatic mosquito stages are encountered both accuracy and the time are vitiated by the human factor.

In July 1954, the author, in an effort to improve upon the time-honored method of collecting aquatic mosquito stages for routine survey purposes by the use of a dipper and eye-dropper, contrived a device, hereinafter referred to as "ADCAS," (euphonic abbreviation of *automatic device* for the *collection* of *aquatic specimens*), which has been found to have merit over the older method. The essential difference between the two methods is that the ADCAS selects and retains from a dipped sample all aquatic mosquito stages *not* smaller than 1/96 inch in size in a matter of seconds, whereas, several minutes or more are normally required to search for and then manually separate with an eye-dropper such aquatic mosquito stages that an individual is capable of recognizing.

The accuracy gained plus the time saved through the use of the ADCAS in performing routine field surveys for aquatic mosquito stages is considerable and has resulted in the complete substituting of the ADCAS method for the eye-dropper method in the Preventive Medicine Survey Section, Ryukyus Command. This section performs a weekly routine survey of 525 stations scattered within an area of approximately 45 square miles on a year-around basis and with the aid of the ADCAS has more than doubled the number of dips normally made previously with the eye-dropper method. Field tests of the ADCAS made in competition with four trained individuals, each with over 10 years experience, demonstrated that an average of 12 per cent of the actual numbers recovered were being inadvertently thrown away after the individual presumed he had removed all mosquito stages from the dipped sample; each man having poured his dipped samples through an ADCAS after he had removed all of the mosquito stages that he could find from the sample. Approximately 23 per cent of the early instars were being overlooked as were 4 and 8 per cent, respectively, of the late instars and pupae representing mixed populations of *Anopheles hyrcanus sinensis*, *Culex tritaeniorhynchus*, *Culex quinquefasciatus* and *Aedes albopictus*. Both individual anopheline eggs and culicine egg rafts were also being retained by the ADCAS.

The ADCAS, figure 1, is constructed of a metal funnel (A), whose larger diameter of 6 inches is soldered into the top of a metal cylinder (B) 7 inches in height and whose smaller diameter of 1 inch is soldered to the metal bottle cap from a 120-cc. aureomycin bottle (C). Two rectangular openings, 1 by 2¼ inches, are made in the side of the funnel and covered with 96-mesh, stainless-steel cloth soldered into place. A circular opening of 7/16 inch diameter is also made in the side of the funnel to accommodate the copper tube siphon (D) which is soldered into place at this junction. A detachable strainer (E) is mounted on the intake end of the siphon and consists of a stainless-steel

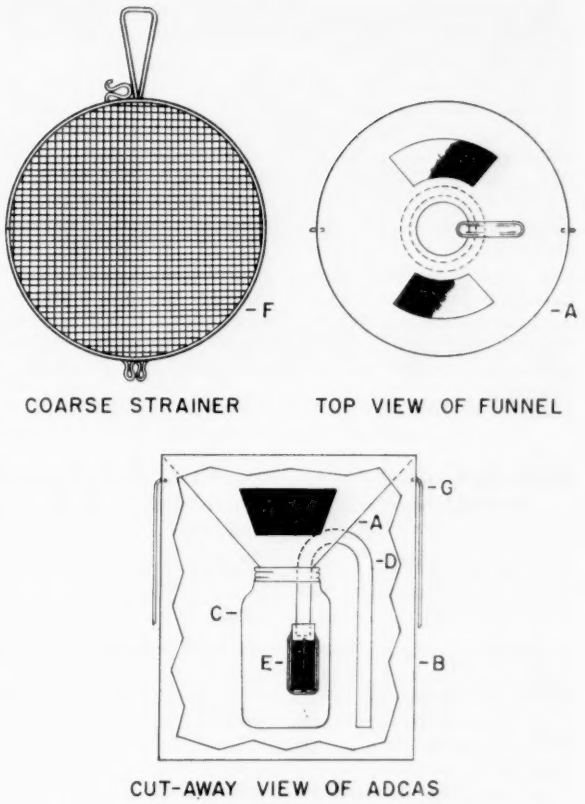

FIG. 1.—Automatic device for the collection of aquatic specimens (ADCAS).

cloth cylinder of 9/16 inch diameter which has its open end soldered to the bronze collar and which is protected by four bronze wire guards soldered into place. The bronze collar is tapped to accommodate the threaded intake end of the siphon. Supporting ends of the 2/16 inch diameter metal bail (G) are inserted near the upper end of the metal cylinder (B). A coarse strainer (F) 6½ inch in diameter is provided for the larger opening of the funnel (A) and is made from 8-mesh galvanized hardware cloth soldered between two supporting metal rings, one ring of which is prolonged and bent to form a handle. Bronze wire keepers are soldered to the coarse strainer for ease of attachment and detachment.

The operation of the ADCAS consists simply of pouring each dipped sample into the funnel (A). The water from the sample flows into the collecting bottle (C), filling it and thereupon filling the siphon tube (D) which automatically discharges and lowers the water level in the collecting bottle (C) to the height of the intake of the siphon (D). Each successive dipped sample will operate the ADCAS in this manner. Upon completing the sampling of a station the collecting bottle with its accumulated specimens is removed, identified and sealed, and an empty collecting bottle is attached for use at the next station.

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[2] Capt MSC, Branch Sixth Army Area Medical Laboratory, Fort Lewis, Wash. The author wishes to express his sincere appreciation to Mr. Joe Poole, Entomologist, Office of the Post Engineer, Fort Buckner, APO 331, San Francisco, California; M/Sgt James W. Gentry, NCOIC Preventive Medicine Survey Section, Ryukyus Command, APO 331, San Francisco, California; Mr. Jose Felicisimo, Assistant Entomologist, Preventive Medicine Survey Section, Ryukyus Command, APO 331, San Francisco, California; and to Mr. Ignacio V. Villanueva, Biological Aide, Preventive Medicine Survey Section, Ryukyus Command, APO 331, San Francisco, California for their encouragement and assistance in this work.

The ¼ inch mesh screening on the coarse strainer (F) is effective in preventing coarse debris, e.g., leaves, twigs, and duckweed, from occluding the entry into the collecting bottle (C) and the 96-mesh, stainless-steel-cloth cylinder (E) attached to the intake retains mosquito eggs, larvae, and pupae and similar-sized components in the collecting bottle (C) while allowing the excess water to escape. Excess water is also permitted to escape by way of the two windows in the funnel (A).

While the ADCAS will accept a certain amount of abuse it will not process water containing an excess of extremely fine particulate matter or an excess of heavy oils. Often, however, even this difficulty can be overcome by tapping and/or shaking the ADCAS in order that the water within the collecting bottle can wash the fine strainer free of occluding debris. It will be recognized by those experienced in this field of entomology that no apparatus can rectify or improve the findings from poor dipping technique.

Mites in Cricket Cultures and the Toxicities of Several Acaricides and Insecticides to the House Cricket[1]

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The house cricket, *Acheta domestica* L., is grown widely in the southeastern United States for fish bait. Commercial cricket growers in Alabama and surrounding states have been concerned for several years by the presence of mites in their cricket cultures. Research was conducted at Auburn in 1953 and 1954 to determine the species of mites that infested crickets and to determine the toxicity of several acaricides and insecticides to the house cricket.

PROCEDURE.—Crickets from cultures from Montgomery and Tallassee, Alabama, and Glenville, Georgia, that were reported to be infested with mites were examined and the species of mites were determined.

Studies were made to determine the toxicities of several acaricides to the house cricket. The following acaricides were used at the indicated rates of technical materials in pounds per 100 gallons of water: Aramite, 0.075, 0.15, 0.3, 0.75, and 1.5; DMC, 0.2, 0.4, 0.8, 2.0, and 4.0; Ovotran, 0.25, 0.5, 1.0, 2.5, and 5.0; and Karathane (dinitro capryl phenyl crotonate and other nitrogen derivatives of phenol, primarily dinitro capryl phenol), 0.125, 0.25, 0.5, 1.25, and 2.5. The crickets were confined in ¼-inch mesh screen wire cages that were 3 by 3 by 3 inches in size. Wettable powder formulations of the acaricides were sprayed at 40 pounds pressure on the crickets with a small portable electric paint sprayer. The crickets were sprayed until they were thoroughly wet, transferred to ½-pint large-mouth jars with screen tops, and provided with water and laying mash for food. Mortality counts were made at 24, 48, and 72 hours after treatment. Each rate of each acaricide and the untreated checks were replicated 4 times with 10 crickets (5 males and 5 females) in each replication.

Experiments were conducted to determine the LD-50 residual deposits of chlordane, endrin, heptachlor, and lindane to the house cricket. The insecticides were dissolved in thiophene-free benzene and sprayed on 6-inch square plywood boards with a spray tower. The crickets were placed in lantern globes and exposed to the insecticides on the plywood boards for 1 hour. Mortality counts were made 24 hours after the crickets were exposed to the insecticides. Each rate of each insecticide and the untreated checks were replicated a minimum of four times; some were replicated as many as 10 times. Each replication consisted of 10 crickets, 5 males and 5 females.

Eleven rates of chlordane were tested, varying from 11.8 to 489.3 μg./cm.² of board. The five rates of endrin ranged from 34.6 to 138.6 μg./cm.² The five rates of heptachlor varied from 2.95 to 29.6 μg./cm.² and the seven rates of lindane from 1.3 to 26.3 μg./cm.² The mortality percentages for each rate of each insecticide were calculated and adjusted according to Abbott's (1925) formula. The adjusted data were plotted on dosage-mortality curves from which the LD-50's were read. The fiducial limits of the LD-50's were calculated according to Snedecor (1946).

Table 1.—Mortality of the house cricket 24 hours after 1-hour exposure to various deposits of chlordane, endrin, heptachlor, and lindane.

CHLORDANE		ENDRIN		HEPTACHLOR		LINDANE	
Deposit	Mortality	Deposit	Mortality	Deposit	Mortality	Deposit	Mortality
μg./cm.²	Per Cent	μg./cm.²	Per Cent	μg./cm.²	Per Cent	μg./cm.²	Per Cent
11.84	0	34.64	0	2.95	4.3	1.32	3.2
16.57	2.1	69.28	5.5	7.80	11.2	2.63	1.1
18.94	4.3	83.15	12.6	14.80	64.5	5.26	35.2
23.68	8.5	103.91	19.7	22.20	73.9	10.53	62.4
26.04	12.8	138.56	71.4	29.60	80.0	15.79	89.7
81.55	44.4	—	—	—	—	21.05	83.5
122.31	53.3	—	—	—	—	26.32	97.9
163.11	59.3	—	—	—	—	—	—
326.22	72.2	—	—	—	—	—	—
407.77	72.2	—	—	—	—	—	—
489.33	77.8	—	—	—	—	—	—

RESULTS.—The mite that was found in the cricket culture from Glenville, Georgia, was *Tyrophagus breviceps* (Banks). The mites from Montgomery were nymphs of a species of the family Acaridae and those from Tallassee were a species of the family Cheyletidae. The cultures and crickets were not studied in sufficient detail to determine if the mites were parasitic and/or predaceous.

None of the four acaricides that were sprayed on the crickets gave definite indications of toxic effects.

The deposits and mean mortalities of crickets following 1-hour exposures to the four insecticides are presented in table 1 and the LD-50's are given in table 2. The percentage mortalities on exposure to chlordane ranged from 0 at 11.84 μg./cm.² to 77.77 at 489.33 μg. The chlordane LD-50 was 100.2 μg./cm.² The mortalities from exposure to endrin varied from 0 per cent at 34.64 μg. to 71.43 per cent at 138.56 μg. with an LD-50 of 128.0. With heptachlor the kill varied from 4.3 per cent at 2.95 μg. to 80 per cent at 29.6 μg./cm.² and the LD-50 was 12.9. The mortality with lindane varied from 3.2 per cent at 1.32 μg. to 97.9 per cent at 26.32 μg. The LD-50 deposit of lindane was 7.8 μg./cm.² There were no significant differences in mortality between the male and female crickets.

Table 2.—LD-50 deposits of chlordane, endrin, heptachlor, and lindane to the house cricket following 1-hour exposures.

INSECTICIDE	LD-50 DEPOSIT IN μG./CM.²	0.95 FIDUCIAL LIMITS
Lindane	7.8	4.0– 13.1
Heptachlor	12.9	12.2– 16.2
Chlordane	100.2	53.0–273.0
Endrin	128.0	113.0–137.0

SUMMARY.—Investigations were conducted in Alabama to determine the species of mites found in cultures of the house cricket. *Acheta domestica* L., and the toxicities of several acaricides and insecticides to this insect. The mite, *Tyrophagus breviceps* (Banks), was found in a cricket culture from Glenville, Georgia. An undetermined species of Acaridae was found in a

[1] The data presented herein are taken from a thesis presented by the senior author to the Alabama Polytechnic Institute in partial fulfillment of the requirements for the M.S. degree. Accepted for publication October 10, 1955.

culture at Montgomery, Alabama, and an undetermined species of Cheyletidae was found in a culture at Tallassee, Alabama.

No toxicity to crickets was exhibited by wettable powder sprays of the following acaricides at the indicated rates (pounds) of technical materials per 100 gallons of water: Aramite 0.075, 0.15, 0.3, 0.75, and 1.5; DMC 0.2, 0.4, 0.8, 2.0, and 4.0; Ovotran 0.25, 0.5, 1.0, 2.5, and 5.0; and Karathane 0.125, 0.25, 0.5, 1.25, and 2.5. The LD-50 deposits with their 0.95 fiducial limits of four insecticides in $\mu g./cm.^2$ on plywood boards following 1-hour exposures of the house cricket were as follows: lindane, 7.8 (4.0 to 13.1); heptachlor, 12.9 (12.2 to 16.2); chlordane, 100.2 (53.0 to 273.0); and endrin 128.0 (113.0 to 137.0).

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A Technique for Studying the Oviposition Habits of the Southern Lyctus Beetle and Its Egg and Early Larval Stages[1]

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The southern lyctus beetle, *Lyctus planicollis* (Lec.) causes extensive losses through damage to seasoned wood products manufactured from ring-porous hardwoods. The species of wood most commonly attacked are oak, hickory, ash, and walnut. The insect attacks and destroys only the sapwood, which contains starch—its principal food.

Initial attack begins with deposition of an egg in the wood by the female beetle. After an incubation period of about 2 weeks the egg hatches into a small larva or "grub" creamy white in color. This stage feeds in the wood, gradually reducing it to a fine powder. In a few months to a year, the larva goes into a resting stage or pupa, the time interval depending on temperature, moisture, and the availability of starch. Within 2 weeks this form changes to an adult, chews its way out of the wood, mates with another beetle and begins a new cycle.

Since egg deposition and development have an important relationship to the evaluation of effectivness of insecticides in control studies, there is a distinct need for being informed on these activities.

During egg deposition the female normally rests on the surface of the wood and extends her ovipositor into a pore opening and along a vessel beneath the surface. Because of this habit, it is not posssible to study the process of oviposition as it naturally occurs; also, under these conditions the egg can be observed only after tedious dissection of the wood. The very delicate egg is usually injured in the process. In an effort to overcome these difficulties in studying the species, a technique has been perfected which permits the oviposition habits and the development of the egg to be studied easily and accurately. This paper outlines the technique and includes a discussion of certain habits and life history details that have been determined through its use.

The procedure begins with the exposure of several springwood vessels on the surface of a piece of hickory wood for about $\frac{1}{2}$ inch along the tangential surface. An area about $\frac{3}{4}$ inch square is then worked down very smoothly to eliminate surface irregularities in the vicinity of the longitudinally exposed vessels. This area is then covered with a thin piece of cover glass which must fit very snugly against the wood. A small drop of household cement along three edges of the glass will accomplish this. The glass-covered vessels retain the two oviposition requirements of the beetle: tubular form and a pore opening to the surface. In addition each has a transparent wall. Once a female is confined to this area, her oviposition habits can be observed continuously, since her activities are restricted to these "windowed" vessels. With such an arrangement, therefore, very accurate observations can be made of the process of oviposition, of the development of the egg, and of the habits of the first instar larva.

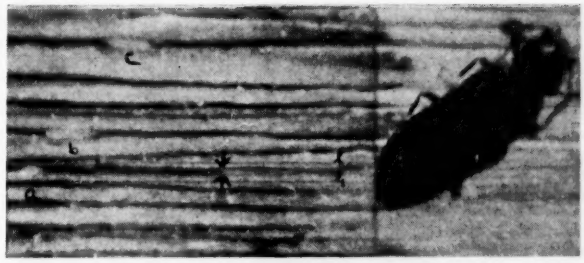

FIG. 1.—Oviposition by *Lyctus planicollis* (× 7).

Figure 1 is an illustration of a female beetle in the actual process of oviposition. The beetle is resting on the surface of the wood at the right and has inserted her ovipositor into a pore opening formed by the edge of the glass and the exposed vessel. The ovipositor extends from the female into the vessel to point (a) where a partially laid egg can be seen. The ovipositor can be seen as a thin thread in the vessel delineated by the two parallel lines 1 and 1_1. Its proximal half is light-colored, the distal half dark. This change in color can be seen at the arrows. Eggs previously oviposited are located at (b) and (c).

Observations have shown that it takes about 20 seconds for an egg to pass completely out of the end of the ovipositor. An additional 25 seconds are spent in disengaging it from the ovipositor. The caudal half of the egg passes out of the ovipositor first by an extruding process. Then with a slight movement of the ovipositor the female attaches the partially laid egg to the wall of the vessel. Once this is done, the cephalic half of the egg is released by a slight withdrawal of the ovipositor rather than by the extruding process. When the ovipositor is not in use, it is held within the body in a telescopic fashion.

This technique permitted accurate measurements of the egg. The length averages 0.87 mm. with a range of 0.64 mm. to 1.21 mm. The width averages 0.16 mm., with a range of 0.13 mm. to 0.21 mm. The egg strand is quite variable in length, 0.07 mm. to 0.42 mm., with an average of 0.22 mm. Thus the average total

[1] Accepted for publication October 17, 1955.

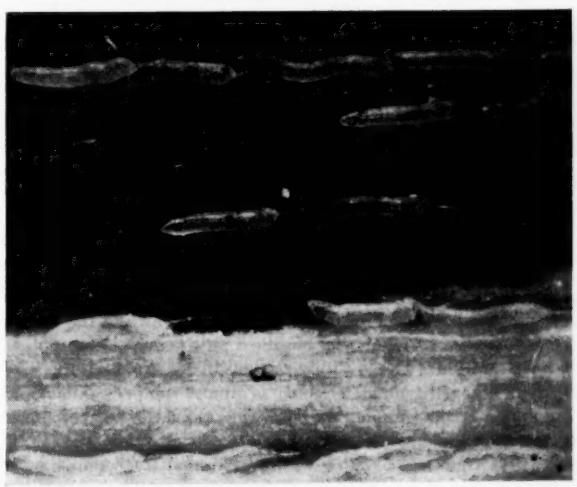

FIG. 2.—*Lyctus planicollis* eggs with natural (a) and blue (b) background (× 20).

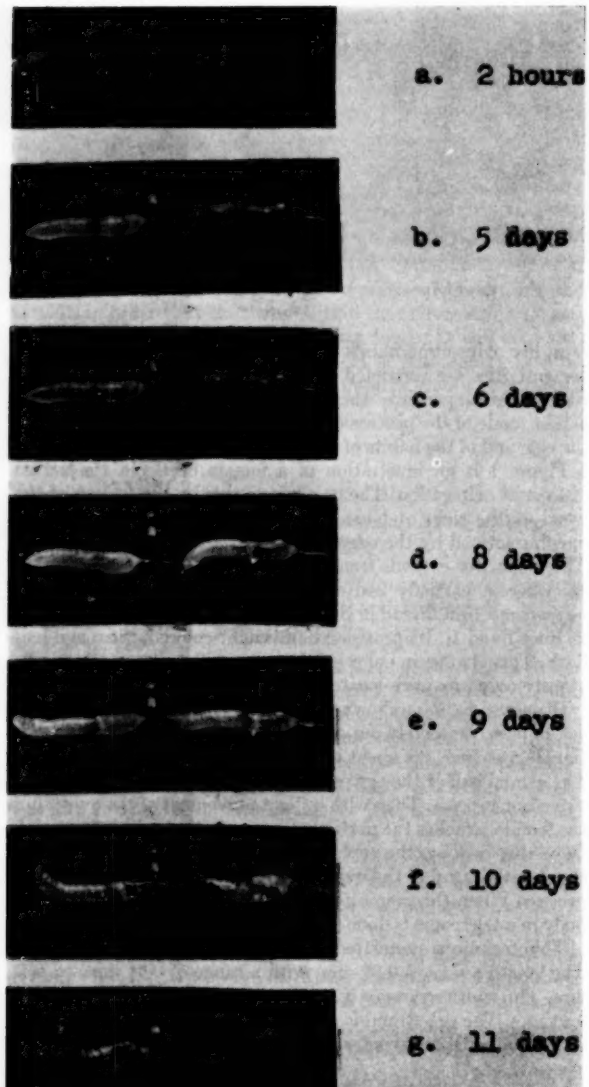

FIG. 3.—Photomicrographs of the development of the eggs of *Lyctus planicollis* after varying periods of time since deposition. (× 20).

length of the egg including the strand is a bit over 1 mm. The eggs may be deposited from 1 mm. to 7 mm. down in the vessel. No extensive data have been obtained on the oviposition potential of the female. The few observations made showed a range of 30 to 80 eggs per female. Viability is almost 100 per cent.

Figure 2 is a photomicrograph of eggs beneath the glass cover with both a natural (a) and a blue (b) background. The beetle was at the right, so that the strand, located at the cephalic pole, is the last part of the egg to leave the ovipositor. The color of the sapwood of hickory is a pale tan, while the egg is creamy white. In order to obtain a clear photograph of the egg in the wood vessels, therefore, it is necessary to modify the wood color. This was done by painting the exposed wood and vessels with blue ink before applying the glass cover. This did not deter the female in oviposition, and it did provide a much better background for contrasting the egg. The characteristic granular appearance of the egg and the parallel longitudinal striae on its surface are evident in figure 2.

An important feature of this technique is that it lends itself to photography. Pictures can be taken at any desired interval to show changes in a given egg or movements of the first larval instar. Some of these, shown in figure 3, illustrate the development of the egg:

a. A slight void is noticeable at the tapered portion of the caudal end.
b. Differentiation within the egg is becoming visible.
c. Differentiation between the cyptoplasm and yolk is becoming more distinct.
d. The larva, now fully developed in the caudal portion of the egg, exhibits movement. There is a slight indication of feeding forward into the yolk at the cephalic end of the egg.
e. Feeding on the contents of the egg is clearly evident. The eggshell at the caudal end has been ruptured.
f. Feeding is accelerated. One larva has passed excreta and changed from a linear to an eruciform shape. It is possible that a molt takes place at this time within the eggshell.
g. All the egg content has been consumed. Both larvae have changed to an eruciform shape and one has turned around in the vessel.
h. (12 days) Larvae have begun to bore into the solid wood adjacent to the vessel. This is usually accomplished by assuming a position perpendicular to the longitudinal axis of the vessel.

There are variations in the time and degree of developments and habits as shown in figure 3, but in general those illustrated are quite typical. One very common habit not shown is movement of the newly hatched larvae some distance along the vessel before entering solid wood.

SUMMARY.—To oviposit, the female of the southern lyctus beetle, *Lyctus planicollis*, rests on the surface of the wood and inserts the ovipositor into a pore opening and down a vessel beneath the surface. A technique has been developed which enables observations to be made easily and clearly and photographs to be taken of the process as well as of the subsequent development of the first instar larvae.

The springwood vessels along the tangential surface of a piece of hickory sapwood are exposed. They are then covered with a thin section of cover glass. This, in effect, creates "windowed" vessels. Female beetles confined to this area oviposit in these visible vessels.

Approximately 45 seconds are required for the oviposition of a single egg. Eight days after oviposition, the larva has completed its development in the caudal portion of the egg, at which time it begins to feed into the yolk in a forward direction. It feeds on the yolk for 2 to 4 days, then leaves the eggshell and enters the solid wood adjacent to the vessel.

Dry Ice as an Insect Anesthetic[1]

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Anesthetization of house flies and other insects for mortality counts has always presented a toxicity hazard to the operator when noxious materials such as carbon tetrachloride or chloroform are used. Bottled carbon dioxide gas eliminates the toxicity hazard but the equipment needed often limits its convenience.

[1] The assistance of Paul H. Eastburg and Bob L. McCaskey in the development of this method is gratefully acknowledged. Accepted for publication November 1, 1955.

An inexpensive and convenient method has been developed by using dry ice to generate CO_2. It is excellent in connection with topical tests using house flies, although it may also be useful for other tests involving anesthetized insects.

A table was constructed with two holes of different sizes. In a 12-inch hole a cylindrical 5-gallon container was placed so that the top was flush with the table; brackets projected toward the inside 2 inches below the top for a removable 16-mesh wire screen fitted to a stiff rim. The vertical distance is critical because the flies have to be deep enough into the CO_2 layer to be kept knocked down but within easy reach of the operator. A smaller hole about 2 inches in diameter contained a metal can, lined with corrugated cardboard for insulation, to hold a small cylindrical wire basket above the ice. Five pounds of dry ice (one-half of a small commercial cake) were crushed in a cloth and placed in the 5-gallon container with the screen removed. No insulation was used around the can so that the room temperature would activate the generation of CO_2 from the dry ice. Dry ice was also placed, to half its capacity, in the small can. Within 5 minutes the large container was filled with a high concentration of CO_2 and continued to generate the gas for about 6 hours.

Water in the bottom of the container for the evolution of the gas is a hindrance rather than an advantage, as it forms a dense fog which has only a low concentration of carbon dioxide. The water eventually becomes so cooled that it cuts down the efficiency of the process. A warm hot-plate may be placed under the container to hasten generation, but it is not necessary in a normally warm room at 70° F.

About 400 flies in a 6-inch cube cage lowered completely into the large container were all knocked down within a minute. The degree of anesthetization can be controlled by the length of exposure. A high concentration of CO_2 is critical since exposure to a weak concentration fails to knock the flies down; instead, it conditions them to resist a stronger or increased exposure.

After the flies were anesthetized the screen was replaced and the flies dumped upon it. One operator prepared the flies for treatment by sorting out the females and placing them in one of the smaller baskets or in the grooves of a 2 by 2 inch piece of corrugated cardboard in such a manner that the same side of each fly was uppermost in order to facilitate the application of the drop of toxicant. The basket or cardboard rested on the large screen until ready for use. When the basket was used, it was finally placed in the small container.

Following treatment the flies were placed in small, cloth-ended recovery cages. The large container of gas may also be used when making mortality counts of cages having large numbers of live flies. In such cases the cage rests directly on the ice for a longer period than described above, to knock the flies out sufficiently for the count, after which they may be dumped directly on the ice for disposal.

During the sexing or other sorting the flies are seldom under the effects of the CO_2 for more than an hour. Any longer exposure may affect a test. When placed in a room above 70° F. they revive in proportion to the length of exposure as follows:

Exposure Time	*Recovery Time*
Up to 5 minutes	1 to 2 minutes
Up to 30 minutes	3 to 5 minutes
Up to 60 minutes	5 to 10 minutes

For quick recovery, as in the vial test of Hoskins and Messenger,[2] where ease of handling is necessary, but where immediate active contact with the residue is required, anesthetization of less than 5 minutes, using small batches of flies is required. Repeating this short knock-down period with a larger batch, as in a stock cage, eventually lengthens the knock down and recovery time.

Dry ice may be obtained locally commercially in the San Francisco area in 10-pound cakes or 50-pound blocks at a cost of not more than 10 cents a pound, although 3 to 5 cents a pound is the more general price. The amount of dry ice required for a 6-hour run thus would not cost over 50 cents. Once the cans are loaded no further attention to the anesthesia is required.

[2] Hoskins, W. M., and P. S. Messenger, 1950. Microbioassay of insecticide residues in plant and animal tissues. Advances in Chemistry Series, No. 1, pp. 93–98.

Oligonychus pratensis, a New Mite on Corn[1]

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In May 1954 the senior author was asked to look at a small field of a dent corn inbred at Weslaco, Tex. The plants had a generally unthrifty appearance, and many of the leaves were reddish, especially toward the ends. Examination with a hand lens showed the under side of the leaves to be covered with fine webs and numerous very small greenish mites. Some of these mites were determined by E. W. Baker of the Entomology Research Branch as *Oligonychus pratensis* (Banks).

The infested field was given a heavy dusting of sulfur with power ground equipment, but this treatment was not effective. When the field was last seen on May 30, about half the plants were dead and the remainder were too much weakened to produce ears. A few infested plants were found in some sweet corn about a quarter of a mile away. On June 1 the junior author also found a 20-acre field of sweet corn and another 20 acres of field corn near La Feria, Tex., badly injured by this mite.

In 1955 this mite appeared to be distributed throughout the Lower Rio Grande Valley, and several fields of dent corn were seriously injured. Many of the heavily infested plants produced little if any grain. Feeding by these mites discolored the husks of sweet corn so much that vegetable shippers rejected the crop from one field of 40 acres even though the ears were perfect.

Specimens of this mite were found on most of the grasses and sedges and many of the weeds in a heavily infested field. Such diverse types as Johnsongrass (*Sorghum halepense*) and careless weed (*Amaranthus retroflexus*) were infested in such fields, although grasses are undoubtedly the favored host. This species was described by Banks in 1912 from specimens collected from timothy at Pullman, Wash. McGregor (1950) mentioned the type slide and species in his revision of the group, and stated that he was leaving the species out of his key because there was no further information available. Females only are found on the type slide.

From reports received during 1954 and 1955 this mite is rather generally distributed in the United States west and south of Kansas, and is a threat to grain sorghums as well as to field and late sweet corn. Early sweet corn may be marketed before the mites become abundant enough to do much harm. Harvey (1954) mentioned this mite (then known as *Paratetranychus pratensis*) on wheat in Kansas and as the most injurious pest of wheat in eastern New Mexico in 1952. It is now recorded from Arizona, Idaho, Florida, Kansas, Montana, New Mexico, Texas, Virginia, and Washington.

E. W. Baker (personal communication) suggests that climatic changes may be responsible for the recent increased incidence of this mite and that drought conditions may be favorable to it. The severest infestations on sweet corn at harvesttime were in those fields that had been dusted with 10% DDT for earworm control, indicating that the use of DDT permits a buildup of this mite in the same manner as it does a mite infestation on cotton.

[1] Accepted for publication November 3, 1955.

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Soap as Spreader and Insecticide[1]

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Soap is widely used in various preparations of sprays of insecticides. It helps the spray to spread uniformly on the plants, particularly on the foliage from which, on account of its smooth nature, the ordinary aqueous insecticide usually slips. However, the quantity of soap recommended for this purpose differs widely from formula to formula and a systematic determination of the percentage of soap needed exclusively for the purpose of bringing about uniform spreading in different plants does not seem to have been made. Besides, although the action of soap as an insecticide itself has been observed in a large number of experiments, in most cases, the information is vague and incomplete because the effect of pure soap solution on different pests has not been compared with that of other insecticides. The authors have, therefore, attempted to determine the value of soap[2] (1) as a spreader on different kinds of leaves and (2) as an insecticide against aphids.

For determining the spreading property, leaves of various plants were dipped in or sprayed with different dilutions of soap. To note its insecticidal value, the insect selected was *Siphocoryne indo-brassicae* Das., the mustard aphid.

RESULTS.—*Determination of the minimum percentage of soap solution necessary for complete wetting.*—Observations were taken with dilutions varying from 0.1% to 2.5%. The results obtained by dipping and spraying the same kind of leaves were more or less similar, except in the case of very fine sprays when a slightly higher concentration (the maximum difference between the two being approximately 0.1%) was needed. Therefore, below only the minimum concentrations found successful in dipping experiments have been given, together with the names of the leaves:

Solanum melongena (brinjal) 0.25%; *Nicotiana tabacum* (tobacco) 0.4%; *Dolichos lablab* (bean) 0.3%; *Lagenaria vulgaris* bottle gourd) 0.25%; *Cucurbita maxima* (pumpkin) 0.25%; *Luffa aegyptioca* (loofah) 0.2%; *Capsicum annuum* (chillies) 0.3%; *Hibiscus esculentus* (lady's finger) 0.3%; *Mangifera indica* (mango) 0.3%; *Psidium guajava* (guava) 0.3%; *Citrus medica* (lemon) 0.4%; *Citrus aurantium* (orange) 0.4%; *Cucurbita ovifera* (vegetable marrow) 0.35%; *Citrullus vulgaris* (water melon) 0.2%; *Triticum vulgare* (wheat) 0.25%; *Hordeum vulgare* (barley) 0.25%; *Pennisetum typhoideum* (pearl millet) 0.35%; *Saccharum officinale* (sugarcane) 0.4%; *Zea mays* (maize) 0.3%; *Pisum sativum* (pea) 0.3%; *Phaseolus mungo* (moong) 0.4%; *Cajanus indicus* (cajanus) 0.3%; *Gossypium sp.* (cotton) 0.3%; *Brassica campestris* (mustard) 0.2%; *Brassica juncea* (rye) 0.2%; *Raphanus sativus* (radish) 0.25%; *Brassica campestris var. rapa* (turnip) 0.25%; *Brassica oleracea var.cauliflora* (cauliflower) 0.4% *Brassica oleracea var. caulorapa* (knol khol) 0.3%; *Brassica oleracea* (cabbage) 0.5%.

Determination of the mortality of Siphocoryne indobrassicae Das.—Spraying was done in a field in January 1953 and the results given in table 1 were recorded 24 hours afterward. Each experiment with a particular percentage of soap consisted of four replications, the fifth in each experiment being performed with plain water.

DISCUSSION.—Different workers have recommended widely different quantities of soap in aqueous insecticides to increase their spreading power. To quote just a few examples, Rahman (1940) in his formula for the preparation of tobacco decoction recommends that 1 part of cheap soap be dissolved in about 40 parts of water. This preparation is to be diluted further with 4 to 5 times of water before spraying, bringing the percentage of soap in the decoction to about 0.5. (in his original formula, Rahman recommends 4 *chhataks* of soap in 10 *seers* of water (1 chhatak=2.061 ozs. and 1 *seer*=16 *chhataks*).

Table 1.—Mortality of aphids caused by different dilutions of soap solutions.

Percentage of Soap	Number of Aphids		Percentage of Mortality	
	Dead	Living	Net	Average
2	24	56	30	29.5
	20	44	31.3	
	12	28	30	
	19	51	27.1	
Check	4	72	5.3	
2.5	25	28	47.1	47.3
	35	36	49.3	
	16	19	45.7	
	12	15	44.4	
Check	2	31	6.1	
3	34	19	64.2	61.6
	26	14	65.0	
	50	34	59.5	
	15	11	57.7	
Check	2	33	5.7	
3.5	22	6	78.6	78.1
	20	6	76.9	
	36	9	80.0	
	54	16	77.1	
Check	4	80	4.7	
4	32	Nil	100	100
	51	Nil	100	
	73	Nil	100	
	88	Nil	100	
Check	3	61	5.6	

In calculating the ultimate percentage of soap in decoctions, consideration has been given to the reduction in the quantity of water on account of evaporation due to prolonged boiling.

Lal (1946) in his formula of *Calotropis* sp. leaf decoction recommends the dilution of soap of 2.5%, ostensibly to act as spreader. Nikolova (1945) uses 2% tar soap as a spreader in gesarol solution to control cabbage moths. Brimblecomb (1947), on the other hand, uses 5% soap solution with creosote to spray timber. With such varied formulae, it is obvious that in some cases the quantity of soap may be insufficient to serve its purpose as a spreader or it may be much in excess of the quantity required and may either be wasted or may even cause damage to the plant. Doubtlessly, different kinds of foliage require slightly different concentrations of soap, but the present experiments clearly indicate that for most varieties of leaves, a dilution of 0.2% to 0.5% is sufficient.

Since the property of a liquid to stick to a particular surface depends upon the surface tension, it may be suggested that any fluid having the same surface tension as that of 0.5% soap will satisfactorily spread on these leaves. The surface tensions of the different dilutions of soap solution found useful are approximately as follows:

0.2%—31.2; 0.25%—31.05; 0.3%—28.14;
0.35%—27.49; 0.4%—27.24; 0.5%—25.23.

[1] Accepted for publication October 11, 1955.

[2] The laboratory analysis of the soap used was: Total alkali 13.68%, fatty acid 79.75%, moisture content 6.06% and insoluble matter 0.51%.

It may, therefore, be generally stated that for effectively spreading on most kinds of foliage, an insecticide should possess a surface tension of approximately 25.23.

There is already substantial evidence on record which proves the insecticidal value of soap solution against small insects, and it appears that the aphids constitute a group which can effectively be controlled by it. Plank (1945) reported that a spray of 4 lbs. powdered soap per 100 U.S. gallons of water reduced aphid population in potted plants by 50% in 4 days. An addition of 8 lbs. of powdered seed of *Mammea americana* and nicotine sulphate (1:800) increased the mortality to 70% and 96%, respectively. He does not seem to have tried to find the effect of higher concentration of soap on these insects. The present experiments show that this dilution is too weak to affect the mortality of *Siphocoryne indobrassicae* to a considerable extent but that 4% soap solution is certainly strong enough to kill the entire aphid population on which it is sprayed. This result is in agreement with the earlier findings of Rahman (1950) on plant lice, who considers approximately 3.5% soap solution to be useful against these insects. He also recommends the same percentage of fish-oil soap for destroying plant lice, citrus sylla, mango-hopper nymphs, etc. In fact, the *Calotropis* leaf decoction recommended by Lal (1946) appears to owe its insecticidal efficiency to the fairly heavy quantity of soap in it. From table 1 it may be seen that a concentration of 2.5% soap alone causes nearly 50% mortality of aphids. Besides *Calotropis* leaf decoction is recognized chiefly as a stomach poison and its value as a contact poison is extremely doubtful. Unless this substance alone can be proved experimentally to destroy a sufficient percentage of these insects, the value of the formula will have to be attributed to the presence of soap in it.

SUMMARY.—The minimum percentage of soap in water for complete wetting of the different kinds of leaves tried varies between 0.2 and 0.5. The percentage required for wetting by spraying is slightly higher than that needed by dipping. This indicates that liquid insecticides having a surface tension of approximately 25.23 will successfully spread on most plants.

If used only as a spreader, soap in excess of 0.5% is superfluous.

A 4% soap solution acts effectively as an insecticide against *Siphocoryne indo-brassicae* Das.

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1954 Studies of Parasites for Sugarcane Borer Control in Louisiana[1]

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The sugarcane borer, *Diatraea saccharalis* (F.), a pest of foreign origin, is the most injurious insect attacking sugarcane in the United States. In Louisiana it causes an annual loss of about 5 million dollars. The possibility of obtaining effective biological control of the borer in the State by introducing parasites that attack it and related species in this country or in foreign countries has been considered in the control program of the Sugarcane Insect Research Laboratory at Houma, La., since 1915. Of the imported parasites that became established, two tachinid species introduced at various times, the Amazon fly, *Metagonistylum minense* Towns., and the Cuban fly, *Lixophaga diatraeae* (Towns.) were the most effective. They were recovered for several years following their release, an indication of their ability to survive the field conditions and winters that prevail in Louisiana. Although promising, these parasites did not increase to a perceptible extent. In 1953, however, additional releases were made, and the parasites became successfully established. Data on releases and recoveries, and a possible explanation of the success in establishment, have been published. These encouraging results prompted a more vigorous program of parasite releases in 1954.

Table 1.—Releases and recoveries of parasites of the sugarcane borer in the Houma, La., area in 1954.

PLANTATION	NUMBERS OF			PER CENT PARASITIZATION BY—	
	Parasites Released		Borers and Parasites Collected		
	Amazon Fly	Cuban Fly		Amazon Fly	Cuban Fly
Valentine Sugars, Inc.	703	—	227	15.5	—
Lafourche Sugars, Inc.	—	1,197	228	—	3.1
Milliken & Farwell, Inc.	750	1,293	241	18.6	4.9
Southdown Sugars, Inc.	1,118	1,559	381	21.1	21.0
South Coast Corp.	1,302	2,692	324	9.4	21.3
Total or average	3,873	6,741	1,461	16.2	12.6

HANDLING OF PARASITES.—Parasite puparia were purchased by the cooperating growers from J. A. Carmichael, Verdant Vale Estate, Arima, Trinidad, B. W. I., at 20 cents each and delivered by air mail to Houma. Upon receipt the puparia were placed in emergence boxes 7½ by 9½ by 12 inches, with three holes in each end into which 1- by 4-inch glass vials were tightly fitted. As they emerged the flies were collected and transferred to cylindrical mating cages 10 inches in diameter and 14½ inches high, covered with light-green organdie. They were fed a mild sugar-water solution and split raisins. To minimize activity and to provide resting areas other than the sides of the cages, a handful of clean green Spanish moss was suspended in each cage. Rearing was conducted in an air-conditioned room, constructed inside a regular laboratory room, with temperatures of 75° to 80° F. and relative humidity about 90 per cent. Cages and moss were sprinkled with rain water several times daily. After a gestation period of 4 to 5 days, the flies were released in the field in areas selected by surveys as having adequate populations of the sugar-cane borer in the proper stage of development. The releases were made at the rate of approximately five parasites per acre.

FIELD TESTS.—From April 19 to July 22, 3,873 Amazon and 6,741 Cuban flies were released on nine plantations in the Houma area. Early in the season 465 Peruvian flies, *Paratheresia claripalpis* (V.D.W.), were released in a single field on another plantation.

Surveys to determine possible establishment and parasitization were made from September 20 to October 21. The Amazon and Cuban flies again became established on all the plantations. No recoveries were made of the Peruvian fly. Practically all known attempts to colonize this species away from its natural habitats and ecological limitations have been failures.

The results of these tests are summarized in table 1. Although the Amazon fly made up only 36.5 per cent of the released number, the average parasitization by this species was 3.6 per cent higher than for the Cuban fly.

[1] These studies were conducted in cooperation with the South Coast Corp.; Southdown Sugars, Inc.; Milliken and Farwell, Inc.; Valentine Sugars, Inc.; Lafourche Sugars, Inc.; the Louisiana Agricultural Experiment Station; and the American Sugar Cane League of the U. S. A. Accepted for publication October 24, 1955.

Table 2.—Recovery of the Cuban fly in 1954 in a field of 1953 summer-planted C. P. 36/13 sugarcane in which the fly overwintered, Houma, La.

Date Collected	Number of Borers Collected	Number of Fly Puparia Recovered	Per Cent Parasitization
January 21	35	4	10.3
February 19	16	0	0
April 27	17	2	10.5
June 7	24	1	4.0
September 9	7	13	65.0
October 4	6	7	53.8
October 29	2	6	75.0
Total or average	107	33	31.2

SPREAD.—Limited surveys to determine the spread of the established parasites were made in the fall of 1954. The Cuban fly was recovered about ½, 1, and 2 miles away from a field in which it overwintered, and the Amazon fly approximately 1 and 2 miles from the nearest release points.

OVERWINTERING OF CUBAN FLY.—In the fall of 1953 there was evidence that the Cuban fly had become established in a field of approximately 75 acres of C. P. 36/13 sugarcane planted late in July and in August, as well as in mature sugarcane, on Woodlawn Plantation at Houma. Accordingly, periodic recovery collections were made in this field during 1954. The data are given in table 2. The January 21 collection consisted of all shoots from five 10-foot samples. On the other dates 1 man-hour was spent dissecting borer-injured stalks and collecting host and parasite material found therein. Since the sugarcane was of a borer-susceptible variety and the field showed a heavy early-season infestation, it was dusted with ryania weekly for 4 weeks beginning shortly after the June 7 collection. No Cuban flies were released on this plantation during the 1954 season.

In a recovery collection made on September 10 in a field of C. P. 36/13 sugarcane ¼ mile away from the first field, 22 borers and parasite puparia were collected, giving a parasitization of 45.5 per cent.

The data on the Cuban fly, including the emergence of adults from the April 27 collection in the laboratory, proved that the species had overwintered. The minimum temperature for the winter of 1953–54 at Houma was 25° F., recorded on February 8. Limited collections indicate that this fly failed to overwinter on the other three plantations where it had become established in 1953.

INCREASED EFFECTIVENESS OF PARASITES IN C. P. 36/13 SUGARCANE.—It appears that parasitization by Amazon and Cuban flies is most effective in C. P. 36/16 sugarcane. This borer-susceptible variety furnishes an ample population of host material, and the borer spends considerable time boring in and out of the stalks and just within the leaf sheaths, thus making itself accessible or vulnerable to attack by the parasite maggots. Another contributing factor could be the availability of food for the adult flies if they feed on mealybug excretions and honeydew from aphids, and presumably they do. Heavy infestations of the gray sugarcane mealybug (*Pseudococcus boninsis* Kuw.), the rusty plum aphid (*Hysteroneura setariae* (Thos.)), and the yellow sugarcane aphid (*Sipha flava* (Forbes)) have been observed in fields of this variety.

EFFECT OF INSECTICIDES ON PARASITES.—Cage experiments were conducted with adult Amazon and Cuban flies of both sexes and of different ages to determine the effect on them of cryolite and ryania, the two commercially used insecticides for borer control. Field conditions were simulated as much as possible. The results were very erratic. Although a heavy mortality of the parasites was obtained when the insecticides were dusted onto the caged plants and flies, there was also some mortality in the untreated checks. Some individuals of both species and sexes survived for 8 days.

The Cuban fly survived the commercial application of ryania for second-generation borer control on three plantations where it had become established, and the Amazon fly survived on one plantation.

MISCELLANEOUS PARASITES.—Four parasites of miscellaneous stalk borers and the pink bollworm, bred at the Moorestown, N. J., laboratory from stock obtained from India, were tested against the sugarcane borer. A total of 22,880 parasites, consisting of 8,707 *Apanteles angaleti* Mues., 8,704 *Bracon brevicornis* Wesm., 4,195 *Chelonus* n. sp., and 1,274 *Chelonus heliopae* Gupta, were released on six sugarcane plantations and in a rice field in the Houma area. Although 588 borer larvae were collected in the fall and winter to determine possible establishment in all the release fields, none of the parasites were recovered.

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Insecticide Tests Against the Three-Cornered Alfalfa Hopper[1]

W. F. CHAMBERLAIN[2]

As an insect pest the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), is generally well distributed over the southern United States. Damage by this insect is consistent but usually is not as great as that of some of the better known alfalfa insects. The primary damage is caused by the feeding or egg laying punctures near the base of the stems. These punctures later cause a stunting of plant growth or the stems may break at the point girdled. The life history, damage, and distribution of this insect were well described by Wildermuth in 1915. Since 1915 the three-cornered alfalfa hopper has received occasional attention by entomologists. It has been the general opinion that this insect is difficult to control but Kulash (1948) and Kulash & Henson (1949) report that control can be obtained by use of 3% BHC–5% DDT dust mixture.

A possible reason why control is sometimes obtained while at other times the insect is difficult to control may be that application of the insecticide dusts or sprays must be applied at the proper time in the life cycle of the insect. Usually during the mid-summer period in South Carolina the populations of the three-cornered alfalfa hopper are very low. During the fall this insect rapidly increases in numbers and occasionally considerable populations may be encountered. In South Carolina four generations of this insect per season are possible and it is the fourth and overwintering generation that occurs in considerable numbers in the fall. Eggs which hatch to produce the fourth generation are laid in July or early August, but at this time attempts to collect the three-cornered alfalfa hopper by sweeping showed very few present in the field. The young nymphs feed at the base of the stems and are very difficult to dislodge into a sweeping net.

Experiments on the control were started during 1955 at a time when no appreciable numbers of this insect were being taken by sweeping. In the first experiment, results of which are recorded in table 1, a small field of alfalfa was divided into 50-foot-square plots. This field had been cut on July 6 and it was treated on July 16, 20, 27, August 13, 19 and 24. Only two insecti-

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Table 1.—Control of the three-cornered alfalfa hopper. First experiment—1955.

TREATMENT	NUMBER OF INSECTS PER PLOT IN 20-SWEEP COUNT[a]					
	July 15	July 23	July 30	Aug. 19	Aug. 26	Sept. 5
DDT 10% dust	—	1	0	0	0	1
Parathion spray	—	0.5	0	0.5	0.5	1.5
Check	0	0	1	4	5	23
DDT spray	—	1	0	1.5	0.5	0

[a] Average of two replicates.

cides were used; DDT as a 10% dust at 20 pounds per acre or as an emulsion at 2 pounds of active ingredient per acre, and parathion as an emulsion at ½ pound per acre. During the first experiment the alfalfa was cut on August 5 and September 8. Insect counts were made using a 15-inch sweeping net with 20 sweeps taken on each plot. The figures recorded in the table are the average number of insects taken from each plot on the dates indicated. At the beginning of the experiment almost no three-cornered alfalfa hoppers were being taken but on September 5 the populations of the check plots averaged 23 per 20 sweeps and the treated plots had less than two insects per plot.

The tests were continued after the September 8th cutting of the alfalfa, but some of the treatments were changed. Untreated plots and those dusted with DDT remained the same. Plots dusted with methoxychlor and toxaphene had been sprayed with DDT in the first experiment and those dusted with malathion

Table 2—Control of the three-cornered alfalfa hopper. Second Experiment—1955.

TREATMENT	NUMBER OF INSECTS IN 20-SWEEP COUNTED—UNREPLICATED						
	Sept. 30	Oct. 7	Oct. 14	Oct. 21	Oct. 28	Nov. 4	Nov. 15
DDT 10%	4	2	1	3	5	0	0
Check	99	108	34	29	10	12	29
Methoxychlor 10%	2	2	3	3	1	0	6
Malathion 4%	2	1	0	0	1	0	5
Toxaphene 20%	0	1	0	0	3	0	0
Parathion 1%	2	2	0	2	1	0	4

and parathion had been sprayed with parathion in the first experiment. The second experiment was designed to determine if insecticides other than DDT and parathion would prevent reinfestation. It was realized that the endemic populations of the insect had in all probability been killed off in the treated plots of the first experiment. Dusts were applied on September 20, 27, October 4, 11, 18, and 25, and the alfalfa was not cut until November 16. The data in table 2 show that a considerable population of this insect developed in the check plot. No reinfestation developed in any of the treated plots during the period of actual treatment but by November 15 a general increase was indicated.

The results of the tests reported here show that the three-cornered alfalfa hopper can be controlled by insecticides, but much more research is needed to develop practical control measures for this insect.

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Biology and Control of Certain Lathridiidae[1]

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Although more than 500 species of Lathridiidae have been described, there is a paucity of literature concerning the biology and control of the family. Hinton (1941) published an excellent account of 30 species of economic importance that have been discovered on stored food products or in granaries, mills or warehouses in Great Britain and presented accounts of the life history and habits of six of the species.

There are few records of the occurrence of the family in Rhode Island. Eddy (1943) reported on the presence of *Microgramme arga* (Reit). From that time to 1954, specimens of *Microgramme costulata* (Reit), *M. arga Cartodere constricta* (Gyll.) and *Adistemia watsoni* (Woll.) infesting houses or warehouses have been referred to the attention of either the senior author or the Rhode Island Department of Agriculture and Conservation.[3] In 1954 assistance in controlling several lathridiids in a large Rhode Island warehouse was sought, the resulting studies being reported herein. During the period of May to December of that year a total of 1,124 specimens, both larval and adult, representing five species were obtained from the warehouse. Of these, 95 per cent were *Adistemia watsoni* while the remaining 5 per cent included *Corticaria serrata* (Payk.), *Microgramme arga*, *Microgramme ruficollis* (Marsh.) and *Cartodere constricta* (Gyll.). Collections from other loci yielded *Cartodere filiformis* and *Lathidius nigritus* (Fall) from the cellar of a disused farm building, *M. costulata* on moldy furniture in the underground portions of a discontinued army fort, *M. ruficollis* in several haymows, and *Adistemia watsoni* from a field of potatoes, the foliage of which was decaying rapidly.

BIOLOGY.—Life history studies of *A. watsoni*, *C. serrata*, *M. arga* and *C. constricta* were completed. All were reared successfully on mixed cultures of *Penicillium*, *Aspergillus*, *Mucor* and *Botrytis* molds. Species of *Penicillium* appeared to be preferred by the insects, the mycelia and conidia being consumed. The molds were grown on moisture-absorbant, rubber substrata that were dipped in Czapek solution and incubated for several days at room temperature under humid conditions.

Since Hinton (1941) and others have described and illustrated the various stages of several lathridiids closely related to some of the species treated in this study, this aspect will not receive attention at this time. It is sufficient to mention that the eggs of all four species were deposited singly in areas of the rubber substrata where mold growth was profuse. The larvae fed actively but were sluggish in movement. Migration was negligible when sufficient mold was present. Preparatory to pupation, third stage larvae attached, ventral side down, by means of an anal secretion at the caudal tip, directly to the rubber substrata. The adults were relatively active. When disturbed physically, or when food was scarce, all species tended to migrate readily. None, except *C. constricta*, was observed to fly.

Table 1 concerns the duration of the various stages of the species indicated. The studies were conducted in the laboratory, the maximum, minimum, and mean temperature being 64° F., 84° F. and 74.6° F., respectively. At 60° F.±2 the average incubation period of eggs of *A. watsoni* and *M. arga* increased to 14.0 and 10.6 days, respectively. Studies on the effect of temperatures lower than that of the laboratory on other stages of these insects were not completed. On investigating the effect of higher temperatures on adult *A. watsoni* it was found that after exposure to 90° F.±2 for 7 days, 4 per cent succumbed. At 100°F.±2, 98 per cent died within 7 days. At 110° F.±2 there was 100 per

[1] Contribution No. 880 from the Rhode Island Agricultural Experiment Station, Kingston. Accepted for publication December 5, 1955.

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[3] Information at the latter source supplied by J. A. Mathewson, Entomologist.

Table 1.—Development of various stages of Lathridiidae at a mean temperature of 74.6° F. Kingston, R. I., 1954.

Species	Duration of Stages (Days)											
	Egg			Larva			Pupa			Egg to Adult		
	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
Cortodere constricta	34	2– 5	3.0	29	4– 7	4.7	16	1– 5	2.3	16	10–16	12.8
Microgramme arga	11	4– 6	4.8	11	3–17	12.3	11	6–10	7.8	9	15–30	24.9
Adistemia watsoni	169	2–11	5.5	141	8–21	13.3	126	6–14	9.6	127	21–35	28.0
Corticaria serrata	38	1– 7	3.5	12	8–13	10.8	10	1– 8	4.3	8	14–26	19.1

cent mortality within 24 hours. At 130° F., 140° F. and 150° F. the time required for complete mortality was 10, 5 and 4 minutes, respectively. The maximum, minimum and mean preoviposition periods of newly emerged females of *A. watsoni* and *M. arga* at a mean temperature of 73.5° F. was 17, 8 and 13.2 and 7, 7 and 7.0 days, respectively.

The larval and adult stages of all species were negatively phototropic. Also, oviposition did not occur and mortality of larvae was increased under conditions of high humidity and condensed moisture in the rearing chambers.

CONTROL.—Although in initial tests DDT, chlordane and dieldrin were effective in controlling adult *A. watsoni* the latter insecticide was selected for further studies. Two formulations were used. One, a wettable powder, contained 50% dieldrin, the other, an emulsifiable concentrate contained 1.5 pounds of dieldrin per gallon. Dilutions of 1 part of the former formulation to 90, 180 and 360 parts of water and 1 to 32, 1 to 64 and 1 to 128 of the latter were used. Each dilution was applied to four pieces of white oak wood 2¾ by 2 by 5/16 inches. Two days after the insecticide was applied, 25 adult *A. watsoni* were placed on the blocks of wood, allowed to remain 1 hour, then removed and placed in a rearing chamber on a piece of moldy rubber substratum and incubated at 75° F. ±2. Their condition as well as that of an untreated group of beetles was observed periodically. Thereafter, from time to time, during a period of 101 days, the pieces of wood were subjected to fresh groups of beetles. These were treated as indicated for their predecessors.

At a dilution of 1 to 90 the wettable powder was 100 per cent effective 101 days after it was applied, while the emulsifiable concentrate at 1 to 32 showed evidence of losing some of its residual effectiveness between 77 and 101 days.

The warehouse in which the various lathridiids had been collected during 1954 was sprayed with wettable 50% dieldrin at the rate of 3 pounds in 30 gallons of water on May 25, 1955. No live lathridiids were found in the structure during the following 4 months.

SUMMARY.—The occurrence of *Cartodere constricta*, *Adistemia watsoni*, *Corticaria serrata*, *Microgramme arga*, *M. costulata*, *M. ruficollis*, *Cartodere filiformis* and *Lathidius nigritus* in Rhode Island is reported. Life history studies at a mean temperature of 74.6° F. showed an average of 12.8, 28.0, 19.1 and 24.9 days required, respectively, for the first four above-mentioned species to complete a generation. All were reared successfully on mixed cultures of *Penicillium*, *Aspergillus*, *Mucor* and *Botrytis* molds, and showed a preference for *Penicillium* species. Adult *A. watsoni* was controlled by DDT, chlordane, or dieldrin, the latter proving effective as a residual insecticide 101 days after application.

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European Corn Borer Attacking Cotton in Missouri[1]

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The European corn borer, *Pyrausta nubilalis* (Hbn), was first observed infesting stalks of cotton in southeastern Missouri on August 9, 1955, in the area of Sikeston. A similar record was reported by Jarvis Moffitt from Franklin County in western Tennessee on July 3 on cotton 8 to 10 inches high. R. P. Mullett, Tennessee extension entomologist, reports that he observed European corn borers infesting an occasional cotton plant 3 or 4 years ago but did not report it at that time. One cotton grower in Missouri reported that he had seen corn borer damage to

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FIG. 1.—Typical appearance of point of entrance of European corn borer into a cotton stem.

FIG. 2.—Mature larva of European corn borer in cotton boll.

cotton in 1954 but this observation could not be confirmed.

Attention was attracted to the first infested stalks observed because of the wilting of the plants above the points of entry which were usually in the upper third of the stalks. The typical appearance of point of entrance is illustrated in figure 1. Later surveys by the junior author determined that a few infested stalks were present late in August in fields throughout the entire cotton growing section of Missouri including the counties of Dunklin, Pemiscot, Butler, Stoddard, Scott and Mississippi. In no case was the infestation of economic importance since the highest stalk infestation in any field surveyed was only 1.2%. The average stalk infestation for the entire cotton growing area of Missouri was slightly under 0.05%.

A few early instar larvae of the European corn borer were found in cotton bolls on August 19 and additional boll damage was observed in early September. Further observations revealed that many second and third instar larvae fed for a period of time on the surface between the bracts and bolls before boring into the bolls. Other third instar and larger larvae bored directly into the bolls causing injury similar to that of the cotton bollworm. Later, some of these infested bolls were found to contain mature larvae (Fig. 2). No fully developed larvae were found in the stems of cotton during September. The borers seem to move about considerably when feeding on cotton stems as was evidenced by numerous empty tunnels in some cotton stalks.

An egg mass of the European corn borer was found on the upper surface of a cotton leaf in mid-August.

Observations on the overwintering potential of the European corn borer in cotton was made on December 17, 1955, in Stoddard, New Madrid, and Mississippi Counties. Borer damaged stalks were split to detect the presence of larvae. Only three fields out of 12 examined were found to have overwintering, fifth instar larvae. The percentage of borer damaged stalks with overwintering borers in these three fields ranged from 1 to 2 per cent.

Further Tests of Lindane in Floor Wax for the Control of Silverfish (Thysanura)[1]

W. E. BECKEL[2]

In a previous communication (Beckel 1955) results of trials to illustrate control of silverfish by the use of floor wax plus lindane were reported. This present note reports the general findings of a series of further experiments designed to discover the effect on silverfish coming in contact with a treated surface only for a short period. Also, an attempt was made to evaluate the effect of lindane where its vapour was removed from the floor to provide more normal conditions than was the case in the experiments reported previously.

MATERIALS AND METHODS.—Tiled plaques similar to those described previously (Beckel 1955) were again used. They were waxed with the experimental liquid floor wax as before, but the silverfish were not placed under a bell jar on the treated tile. It was suggested to the author that the vapour from the lindane trapped in the bell jar might have contributed to the mortality of the insects. Therefore, 3-inch cylinders of glass, open at each end, were used to enclose the silverfish on the tiles; a fan circulated the air above the cylinders.

Experiments were performed to test the knockdown of silverfish after they were exposed to the freshly waxed tiles for a definite period then removed to an untreated surface. The insects were exposed for 30 seconds, 3, 6, 12, 18, 24 minutes. Concentrations of insecticide at the 1% and 5% levels of lindane in oil plus wax and wettable powder plus wax were used. The experiments also included leaving the insects on the treated surface until they were intoxicated. Tile freshly treated and also left for 7 days was tested. Ten silverfish were used in each experiment. An insect was considered intoxicated when it had turned on its back and stopped moving.

RESULTS.—Only general conclusions drawn from the data will be presented. Tables of insecticide effect will not be given as considerable recovery after initial intoxication was recorded. Experiments to record actual death of the insects have not been done.

With either 1% or 5% lindane in oil or as powder, exposure of over 24 minutes was necessary for 50% knockdown. In some cases the symptoms of intoxication did not appear until 3 days after exposure. Controls run concurrently showed no such symptoms. In some experiments where the insects spent less time on the treated tile, 50% initial intoxication resulted but there was considerable recovery of the insects.

The experiments where the insects were allowed to remain on the treated tile until death showed that indeed the experiments reported previously (Beckel 1955) were unreal. Probably the trapped vapours of lindane in the bell jar did kill many silverfish. When 10 specimens were placed and remained on a freshly treated tile in a 3-inch cylinder with a fan blowing over the open top, it took up to 1 hour to intoxicate all the insects. No more rapid effect was observed when 5% lindane was used. When tiles were left for 7 days after treatment and the 10 insects were

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placed on them, all were on their backs only after 20 to 24 hours; again the 5% lindane produced no more rapid effect. No recovery of the insects in these experiments was noted.

It is concluded that the efficacy of lindane in floor wax to control silverfish is not as great as indicated by the results of earlier experiments.

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Overwintering Habits of the Geminate Leafhopper in Utah[1]

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In addition to its earlier known role as a vector of aster and celery yellows (Severin 1934), the geminate leafhopper, *Colladonus geminatus* (Van D.), has in recent years become of major importance as a vector of other viruses, such as the western X-disease in peach, cherry, and chokecherry in Washington, Oregon, and Utah (Wolfe *et al.* 1950, 1951; Kaloostian 1951; Nielson & Jones 1954). It has been reported as a vector of the yellow leaf roll disease, or strain of western X-disease, of peach (Jensen *et al.* 1952), and the Napa strain of the cherry buckskin virus in California (Jensen & Thomas 1954). Its life history on virus-infected and healthy greenhouse celery plants was reported by Severin & Klostermeyer (1950), but no information has been available on the overwintering habits of this leafhopper in the field. This information is desirable before a complete control program can be planned for the insect.

In September 1953 a study was undertaken at Logan, Utah, to determine the overwintering habits of the geminate leafhopper in this region. Approximately 6,000 greenhouse-reared leafhoppers were used in the experiments as follows:

(1) Cold storage chamber: About 250 leafhoppers were placed in nylon sleeve cages on each of four potted mazzard cherry trees, and kept in a walk-in cold-storage chamber at 34° F.

(2) Screenhouse: Same as in (1) except that the leafhoppers and trees were held in an outside screenhouse.

(3) Plot trees: Same as in (1) except that the leafhoppers were caged on mazzard cherry trees at the experimental field plot near Logan.

(4) Plot trees: About 2,000 leafhoppers were released into each of two overwintering cages (27 cubic feet) made of 16-mesh screen at the experimental field plot near Farmington, Utah. Each cage contained five small peach trees and newly planted alfalfa.

(5) Greenhouse: Supplementary observations were made on about 1,000 leafhoppers feeding on celery and alfalfa in rearing cages in the greenhouse.

A noticeable reduction in the number of leafhoppers occurred in October in all cages including those in the greenhouse. Mortality of leafhoppers increased as their food became less attractive. All leafhoppers in the field cages and those in the cold-storage chamber were dead by December. By this time heavy frosts had occurred in the field. In the greenhouse an appreciable number remained alive through December, and a few individuals used in virus-transmission tests survived through March and early April. It had been determined perviously that under favorable conditions adults could survive more than 7 months.

Oviposition was observed through October and November. Indications were that this leafhopper would pass the winter in the egg stage in the field. In the culture cages eggs were inserted in the leaf and stem tissues of alfalfa and celery plants. Leafhoppers caged on cherry and peach trees oviposited in the leaves; no eggs were found on the twigs and buds. Counts on the cherry leaves showed that, of 500 eggs, 440 were inserted on the lower surfaces and 60 on the upper surfaces. The edges of the leaves were the most favored areas of oviposition.

Autumn-laid eggs on celery and alfalfa began to hatch in the greenhouse cultures in November. Hatching reached a peak in December. Eggs laid in autumn on cherry leaves and exposed to 34° F. for 30, 48, 50, and 120 days in the cold-storage chamber began to hatch within 7 days after being transferred to the greenhouse where the temperature ranged between 70° and 80° F. Eggs subjected to outdoor temperatures as low as −10° F. began to hatch in April. Examination of leaves from a cage in the field plot on April 13 revealed the presence of first instar nymphs, indicating that hatching had started on or before that date. Some of these eggs and nymphs were placed on alfalfa in a cage in the greenhouse, and the first adults appeared on May 20. First adults were observed in the outdoor cages on June 1, in the field outside the cages at the same location on May 19.

It is apparent from these observations that the geminate leafhopper overwinters in the egg stage in Utah. No definite rest period or dormancy was required for hatching; cold temperatures merely delayed development. Eggs inserted into leaves on trees survive after the leaves have fallen. On alfalfa eggs have survived in stem tissues and retained leaves. It has been known that this leafhopper can be reared continuously in the greenhouse.

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[2] In cooperation with the Utah State Agricultural College and Experiment Station at Logan, Utah.

Mosquitoes in Farm Ponds in Maryland[1]

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During recent years the number of farm ponds in Maryland has been increasing. The creation of small impoundments is promoted by conservation agencies for various purposes such as prevention of run-off, irrigation, watering of livestock, and fish production. The presence of these ponds creates a possible public hazard when they are located on farms adjacent to towns or industrial centers in rural communities.

A survey in five Maryland counties was made during 1955 to determine whether or not typical farm ponds are an important source of mosquitoes. The Soil Conservation Service of the U. S. Department of Agriculture cooperated by supplying information about the history of the ponds and help in locating them. A random selection of ponds was made so that ponds of different size and age in various parts of the five counties would constitute a representative sample. The ponds ranged in size from 0.5 to 5 acres (average 1.67) and in age from 1 to 10 years. The ponds which the Soil Conservation Service assists in building must not exceed 1.3 **acres.**

Initial visits were made on June 14 intermittently through July 14. During this period 55 ponds were studied. Larval populations were sampled by dipping around the edges of the ponds, the number of dips at each pond ranging from 30 to 100 depending upon the size of the pond. Data were obtained on the type and amount of vegetation and the presence of fish in each pond. About 6 weeks after the first visit 30 ponds were visited again from August 23 through August 25. Ponds selected for a second visit were chosen at random without regard to their condition at the time of the first visit. Heavy rains occurred about 10 days prior to the second visit, and the water level in all ponds was higher than at the time they were first observed.

Table 1 shows the number of ponds in each county where larvae were found. In those ponds which were positive the average number of larvae per dip ranged from 0.014 to 10. There were four ponds in which the average number of larvae per dip exceeded 1. Table 2 gives information about these ponds. If the perimeter of the pond had emergent vegetation to the extent of one-third or less, vegetation was considered slight; if from one-third to two-thirds, medium; and if two-thirds or more, heavy. In the case of the pond which was stocked with fish between visits there seemed to be little doubt about the beneficial effect of introducing the fish. Considering all 55 ponds it appeared that when fish were present larvae were absent or occurred in very small number. There appeared to be little correlation between presence of larvae and amount of vegetation.

Species of larvae collected were *Culex territans* Walker (apicalis of authors), *C. restuans* Theob., *C. pipiens L.*, *Anopheles punctipennis* (Say), and *A. crucians Wied.* Of these, *Culex pipiens* is the only species which can be considered an important pest mosquito in Maryland, and it was found in only one pond. On the whole, larvae were found in relatively few ponds and in relatively small numbers. Most of the species collected were those which do not readily bite man.

As a result of this survey it may be concluded that up to the present time farm ponds examined in the five Maryland counties are unimportant sources of pest mosquitoes. It is expected that ponds in other counties will be sampled in 1956.

Table 1.—Collections of mosquito larvae from ponds in five Maryland counties, 1955.

County	No. of Ponds Visited	No. Positive	
		First Visit	Second Visit
Anne Arundel	18	7	2
Prince Georges	6	1	0
Montgomery	13	0	1
Howard	6	1	0
Frederick	12	0	0
Total	55	9	3

Table 2.—Conditions in four Maryland ponds studied in 1955.

Average No. Larvae per Dip		Age of Pond in Years	Size in Acres	Vegetation	Fish
First Visit	Second Visit				
1.3	0	5	4.0	Slight	Present
3.0	0.02	10	0.8	Heavy	Absent
8.0	.07	2	.6	Medium	Absent
10.0	0	1	1.0	Heavy	Stocked between visits

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Control of the Taxus Mealybug with Notes on Its Biology[1]

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In June 1955, attention was called to a severe infestation of mealybugs on *Taxus cuspidata* in Northampton, Massachusetts. Specimens of the insect were identified by Dr. H. Morrison of the Entomology Research Branch, U.S.D.A. as *Pseudococcus cuspidatae* Rau. Apparently this is the first published report of its occurrence in Massachusetts. Twelve mature trees about 18 feet high and 15 feet in diameter, as well as several small shrubs and a hedge, were attacked so severely that the foliage was sparse with some yellowing. The needles were caked with sooty mold and honey-dew. A weakening of the entire tree occurred in nearly every case to the extent that there was very little new growth. Branches, twigs, and new shoots throughout were nearly covered on the underside with mealybugs. Because this species clusters and feeds on the underside of initially dense branches, an infestation may not be observed readily, particularly by laymen, until the plant is seriously weakened. Plantings of yew should be checked periodically for white masses of mealybugs in the crotches of larger twigs and branches in order to detect the pest before a serious infestation occurs.

A survey of the literature revealed a paucity of references to *P. cuspidatae.* Weiss (1915) first recorded the pest from a New Jersey nursery on *Taxus* plants originally obtained from Japan. It was identified and subsequently referred to as *P. kraunhiae* Kuwana until Rau (1937) described it as a new species, *cuspidatae.* According to Rau, it has been recorded on *Taxus* in New York, Connecticut, and New Jersey. Several authors have suggested that it probably occurs widely through the Northeast.

The only studies on biology and control of *cuspidatae* known to us are those of Hamilton (1942). Two other species also occur on *Taxus.* Neiswander (1949) studied *P. maritimus*, the grape mealybug, on *Taxus* in Ohio and stated that *cuspidatae* did not occur there. *P. comstocki* has been reported attacking yew commonly (Hamilton 1942). All three are similar in life history except

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that *cuspidatae* is viviparous; the other two species lay eggs (Hamilton 1942). There are at least two generations a year in New Jersey and probably two in New York, Connecticut, and Massachusetts. In New Jersey the winter is passed as first stage nymphs in bark crevices and the adults are present in June and August.

We found both nymphs and adults during June but no eggs or young nymphs. Apparently the life cycle pattern is similar to that in New Jersey. Following the application of insecticides, yellow masses of various sizes were found on the branches. They were made up of oval egg-like structures and it is speculated that they were partially developed mealybugs. Dissection of some females showed the presence of small elongate ova. It is necessary to make further detailed studies to determine what phenomena take place in the production of young. Hamilton (1942) reported that no ovisac was produced in *cuspidatae* and that the young are born alive. Attempts to colonize mealybugs on young plants in the laboratory were unsuccessful and no mealybugs were found on the plants in the field after they were sprayed.

Recommendations for control of mealybugs on *Taxus* have ranged from the use of nicotine and soap (Hamilton 1942), lauryl thiocyanate (Neiswander 1949), to the more recent materials, parathion and malathion (Boyd 1953, Matthysse 1954). Little has been published on the effectiveness of some of the newer insecticides against the taxus mealybug. With the difficulty of obtaining thorough wetting, one formulation or combination of materials may be more effective than another. Malathion has shown considerable promise against mealybugs on other plants and is much less hazardous to use than parathion. Thus we conducted some tests to determine: (1) the effectiveness of various formulations of malathion; (2) the effectiveness of various formulations of BHC; (3) the effectiveness of a formulation of DDT and malathion in combined form (Blackleaf 258); (4) the influence of the addition of Triton X-100 on the effectiveness of wettable powder sprays; and (5) the likelihood of obtaining complete control.

Sprays were applied with a Spartan "estate sprayer" which was operated at about 225 p.s.i. Particular care was taken to spray upwards and out from beneath the branches to obtain thorough coverage. A single tree was used for each treatment and received from 12 to 15 gallons of spray mixture. A total of 10 trees included a check which was not sprayed. Prior to treatment we counted the number of mealybugs on an average of 8 to 10 inches of branch at three places around the periphery of the tree. Individuals on new growth and second year wood were observed. It was not possible to obtain accurate counts where the mealybugs were more abundant because they clustered together several layers thick. At 2- and 7-day intervals following treatment, the number of mealybugs found on those same twigs was recorded as shown in table 1. Some variation in counts is due to the movements of some mealybugs to and from the twigs. The force of the spray and the handling of the twigs to make counts seemed to disturb a few of the mealybugs somewhat.

From our preliminary tests it is readily apparent that malathion is effective against the taxus mealybug. Malathion emulsifiable concentrate used at the rate of 2 pints per 100 gallons gave the most rapid kill, although at the rate of 1 pint and in combination with DDT (Blackleaf 258) as a wettable powder, equally effective control was obtained. At the rate of 2 pounds per 100 gallons, 25% malathion wettable powder did not eliminate all of the mealybugs after a week, even with Triton X-100 added, although considerable reduction was effected. BHC as used in these tests was not appreciably effective.

Because it was not feasible to maintain any mealybugs as checks, we attempted to eliminate the insect from the plants. One week after the treatments were applied, all yews were sprayed either with 57% malathion emulsifiable concentrate at the rate of 1 quart per 100 gallons or a combination of 25% malathion and 50% DDT wettable powders each at the rate of 3 pounds per 100 gallons. Applications were made with a large power sprayer operated at 600 p.s.i. During July and August we were unable to find any live mealybugs, even on a few small plants which were not sprayed quite so thoroughly as the others. Although we did not demonstrate a definite prolonged toxic effect of the malathion-DDT conbination, the lightly sprayed plants indicated that such an effect is likely and may compensate somewhat for the difficulty in obtaining thorough wetting. Nevertheless, it is vitally important to insure maximum coverage and wetting in controlling this pest. Taxus plants usually have dense foliage which protects the mealybugs. Furthermore, commercial spraying, under most conditions, is less thorough than experimental applications.

Table 1.—The effect of certain insecticides on the taxus mealybug.

Materials Used per 100 Gallons of Water	Total Number of Mealybugs[a]		
	Before Treatment	After Treatment	
		2 Days	7 Days
Malathion E. C.[b] (47%), 2 pts.	272	0	0
Malathion E. C. (47%), 1 pt.	277	8	0
Malathion W. P.[c] (25%), 2 lbs. and DDT W. P. (50%), 4 lbs.[d]	450	7	0
Malathion W. P. (25%), 2 lbs., and DDT W. P. (50%), 4 lbs.[d] plus Triton X-100, 2 oz.	400	7	0
Malathion W. P. (25%), 2 lbs.	338	42	14
Malathion W. P. (25%), 2 lbs. plus Triton X-100, 2 oz.	290	72	27
BHC E. C. (25% gamma), 1 pt.	295	238	211
BHC W. P. (25% gamma), 1 lb.	524	391	403
BHC W. P. (25% gamma), 1 lb., plus Triton X-100, 2 oz.	368	253	256
Untreated	100	83	91

[a] Number of mealybugs observed on three tagged twigs per tree.
[b] Emulsifiable concentrate.
[c] Wettable powder.
[d] Blackleaf 258

Summary.—A severe infestation of the taxus mealybug, *Pseudococcus cuspidatae*, was eliminated by the application of sprays containing malathion emulsifiable concentrate or a combination of malathion-DDT wettable powder. Formulations of BHC were not effective in controlling this pest.

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Distribution and Habits of the Giant Hornet in North America[1]

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The common hornet of Europe, *Vespa crabro*, has several varieties of subspecies which can be distinguished on the basis of color. Bequaert (1931b) indicated that the form introduced in to North America is the giant hornet, *Vespa crabro germana* Christ, which differs from the typical variety by having extensive reddish or orange markings on the thorax. In *Vespa crabro crabro* the thorax is almost completely black.

The exact date of the introduction of this large and showy insect into North America is not known. Bequaert (1955) summarized the early records of its occurrence in the New World. He indicated that the hornet was reported in the vicinity of West Farms, New York, about 1840 and that De Saussure stated that specimens were collected in eastern United States in 1854; also that Packard reported the presence of the insect near Morristown, New Jersey, in 1863 and that Du Buysson indicated that a specimen had been taken in New Orleans in 1867. Bequaert stated (1931b) that the giant hornet was thoroughly naturalized on Long Island and Staten Island, in southeastern New York as far north as Poughkeepsie, northern New Jersey, southwestern Connecticut, eastern Pennsylvania, Maryland and Delaware. The same investigator (1941) indicated that specimens had been taken in North Dakota and the province of Quebec. Wing (1949) indicated that the giant hornet had spread to North Carolina, Ohio and West Virginia.

Bequaert (1955) indicates that he had seen a specimen from Ithaca, New York taken in 1936. Collins (1955) presented data which supports his belief that the insect had reached the vicinity of Albany, New York about 1933–34. However the first authentic collection from Albany was not made until 1952. Specimens from the collection at the University of Massachusetts bear the data Newport, New York, 1948. Evans (1955) states that the insect is taken regularly in Ithaca, New York and a number of infestations in houses have been reported in that area since 1953. Miller (1955) stated that nesting of the giant hornet has been reported from Montreal and St. John, Quebec. Kerr (1955) reported that a specimen from the collection at the University of Rhode Island was taken in a greenhouse in Kingston, Rhode Island, in 1922. Krombein (1955) observed specimens of this insect in Marietta, South Carolina.

The attention of the authors was drawn to this insect following reports of injury to lilac in Williamansett, Massachusetts. We visited the area and collected about 50 specimens from lilacs. we were not able to locate the nest even though we searched the area for several hours. Subsequently we observed a nest which had been found in an empty nail keg in Hatfield, Massachusetts. This nest consisted of several horizontal combs but lacked any surrounding paper covering.

Subsequent investigations indicate that the hornet became established in these areas in Massachusetts about 1952–53. A specimen in the collection at the University of Massachusetts bears the data "Amherst, Massachusetts 1952." However this was taken by a student and may not constitute a valid record.

HABITS OF THE GIANT HORNET.—Beutenmuller (1898), Felt (1915), Cory (1931) and Wray (1954) have made observations on the nesting habits of this insect. Apparently this species, at least in this country, does not usually build its nest in the open. Reports indicate that hollow trees, hollow posts, sheds, barns, porches and even attics seem to be preferred nesting sites.

Cory (1931) fumigated a nest in early October. He recovered 29 queens, 142 workers, 116 males, 95 pupae and 31 larvae from this nest. Wray (1954) reported nests varying from 2 to 3 feet long and 10 to 20 inches in diameter. Many authors have reported various types of damage caused by these insects. They may girdle the smaller twigs and gnaw holes in the bark of the larger branches. We noted damage to lilac and syringa. Other observers have reported that this wasp injures birch, ash, horsechestnut and dahlias. There is also evidence that it attacks various fruits nearing maturity.

There appears to be a lack of agreement as to the purpose of the injury to twigs and branches. Some believe that the hornets are seeking nest materials, others believe that they are primarily interested in securing sap. An interesting observation was made in relation to the gnawing of the larger branches. In each case, larval lilac borers were found within such areas. Whether there is any association between the presence of the borers and the hornets is unknown.

As a result of numerous observations we formed the opinion that the adult giant hornets, away from the nest, were not particularly vicious. Even after they were disturbed, the insects appeared to be more curious than angry. They would hover in front of us but they made no effort to sting. Dissections revealed the presence of a particularly long sting and a large poison sac.

SUMMARY.—The first record of the presence of the giant hornet in Massachusetts is recorded. Records indicate that the insect is well established in parts of Quebec. The hornet has continued to spread northward and westward in New York state. Krombein recorded its presence in South Carolina and Kerr indicated that it was taken in Rhode Island in 1922.

Although we have contacted all probable sources of information, we have no records of the establishment of the insect in eastern Massachusetts. This is somewhat surprising since it might be expected that it would be found on Cape Cod.

According to Dr. Bequaert, reports of the occurrence of *Vespa crabro germana* in North Dakota and Louisiana should be considered as accidental. We have no evidence of its establishment in either of these states.

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[1] Accepted for publication January 6, 1956.

The Biology of *Ferrisiana virgata*, a Pest of Azaleas[1]

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The discovery of a heavy infestation of the mealybug, *Ferrisiana virgata* (Ckll.) (Pseudococcidae; Homoptera), on azaleas was responsible for this investigation of the life history and habits of this pest. *Ferrisiana virgata* was first recorded in 1893 in Jamaica (Cockerell 1893), and described the same year (Cockerell 1893a). The first record of occurrence in the United States was from Texas in 1895 (Howard 1895), and since then it has been reported as occurring throughout the tropical and subtropical regions of the world. Published common names for this mealybug are the striped mealybug (Merrill 1953), the white lamtoro mealybug (Begemann 1929), and the common mealybug (Das 1948).

DESCRIPTION.—Ferris (1950) gives a complete description of the adult and the synonomy. The following observations were made on specimens reared in a greenhouse:

Egg: oval. 0.34 mm.×0.17 mm. Buff to light yellow in color. The eggs hatch in a very short time after oviposition and are therefore seldom seen.

Nymph: 0.34 mm. long at hatching. Light yellow at hatching. Moderately active. Six antennal segments in first instar, 6 in the second instar, 7 in the third instar and 9 in the fourth instar of the male.

Adult male: 1.16 mm. long exclusive of caudal tassels, which extend beyond wing tips at maturity. Winged but never observed to fly. Ten antennal segments.

Adult female: 1.19 to 2.72 mm. exclusive of caudal tassels just prior to oviposition. Moderately to heavily covered with a dusty white secretion except for three small sub-medial patches on each side of the dorsum. These patches are bare and correspond to areas which are devoid of trilocular pores (Ferris 1950). Glassy threads of wax which curve gently arise from the dorsum. These may attain a length of several times the length of the body. Stout tassels of waxy secretion arise from the anal lobes. These averaged 0.85 mm. for 10 individuals. The lateral tassels which are common in the mealybugs are lacking in this species.

HOST PLANTS AND DISTRIBUTION.—*F. virgata* may be described as being generally but not exclusively a tropical and subtropical insect, having been recorded from all over the world in tropical and subtropical regions. However, its presence in Maryland indicates that it is capable of survival in colder climates, and thus it must be considered a potential pest in temperate areas.

The list of recorded host plants is extensive. A review of the literature indicates that this pest is capable of survival on almost any flowering plant. It has been reported injurious to jute (Das 1948), and coffee (Begeman 1929), a minor pest on citrus (Wollcott 1936) and destructive to cotton (Smythe 1920). It has been implicated in the transmission of the swollen shoot virus of cocao by Strickland (1950) and Posnette (1947). This is one of the rare instances of a virus being transmitted by a member of the family Pseudococcidae.

LIFE CYCLE UNDER LABORATORY CONDITIONS.—For this phase of the study, 6-inch potted azalea plants were used. These were maintained in a greenhouse where the maximum temperature was about 95° F., and relative humidity 65%. In order to isolate individuals for daily observation, 1- to 3-inch apical sections of twigs were isolated by encircling the twig with absorbent cotton, securing it with adhesive tape and smearing the barrier with petroleum jelly. In obtaining newly hatched individuals with which to infest the twigs, gravid females were collected from the natural host, returned to the greenhouse and examined daily until nymphs were produced. Thus each individual used in this phase was less than 24 hours old when isolated on the twig. Nymphs were removed from under the adult with a soft moistened brush. Observations and records were made daily until oviposition.

Table 1.—Average duration of nymphal instars of *Ferrisiana virgata* reared under laboratory conditions.

INSTAR NUMBER	AVERAGE DURATION (DAYS)	RANGE (DAYS)	NUMBER OF OBSERVATIONS
	Female		
1	10.6	8–14	21
2	8.9	7–11	17
3	9.3	8–11	17
	Male		
1	13.3	11–13	6
2	15.8	11–18	5
3	3.6	2–6	5
4	5.3	5–6	3

Within 2 days after hatching the white powdery secretion and short waxy dorsal threads began to appear. At the same time, caudal tassels about half the length of the body developed. Dorsal threads are present after each molt. Immediately after the last molt the female is greenish-brown, with lateral margins of the dorsum distinctly brown. Within hours the female becomes entirely brown, then grey as the white dusting appears. First instar nymphs usually feed at the bud tips, or less often at the leaf axils. As they matured feeding took place at the bud tips and main stems. Only occasionally did feeding take place on the leaves, and this was almost exclusively on the leaf stem at the axil.

DURATION OF IMMATURE STAGES.—Results were recorded and tabulated only when the period between molts was noted with utmost certainty. Thus the average for the duration of the stadia was based on varying numbers of observations. Table 1 gives the results of these observations.

Females molted three times, requiring an average of 28.8 days to reach the adult stage, while males molted four times and required an average of 38 days to reach the adult stage. The period from egg to oviposition ranged from 42 to 56 days in 10 females, with an average of 51 days.

The males moved to a protected place after the first molt, and about 3 days before the second molt began to form a flimsy cocoon by the recurving of the dorsal filaments. The cocoon was completed in about 3 days; and while in the cocoon, the males molted three times. After the first molt within the cocoon the males were dark brown and were greatly altered in general appearance, having begun to assume the form of the adult. Wing buds were present at this time. The caudal tassels began to form within hours after the last molt, and in about 3 days they extended beyond the wings. The male left the cocoon about 41 days after hatching. Copulation was never observed, though several times males were observed in close association with females. Das (1948) reported that males are scarce and that parthenogenesis is common.

SEASONAL CYCLE IN MARYLAND.—Continuous field observations were made on large Hinodigiri azaleas for a period of 17 months beginning in June, 1954. These plants were heavily infested during the first summer of observations, but the population declined sharply during the second summer.

The nymphs left the overwintering site about the first week in April and began to feed at the axils of old and new leaves and at the bases of flower buds. After a period of about 3 weeks the females began to construct peculiar nests beneath their bodies.

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These nests appear to be constructed of tattered fragments of the glassy wax threads arising from and recurving around the dorsum.

The first nymphs of the new generation appeared during the second week in June. The young nymphs fed on tender growth, especially on the flower buds, but as they matured feeding took place on all parts of the plants except the roots and main stem.

The next generation appeared about the first week in August. These are the overwintering forms, and with the advent of cooler weather these nymphs ceased feeding and moved to empty seed capsules which were produced the previous year. Here the second or third instar nymphs secreted themselves and passed the winter. Attempts to determine the exact overwintering stage failed when the mealybugs that were collected for this purpose died because of parasitization.

In Maryland this mealybug is not attended by ants, there being a complete lack of honeydew. Merrill (1953) states that in Florida *F. virgata* secretes large amounts of honeydew. Presumably this is governed by the host plant.

PARASITES.—It is probable that natural control by parasites is an important factor in keeping *F. virgata* from becoming a pest of economic importance in many areas. A high rate of parasitization was noted in overwintering forms. The following parasites were found in Maryland:

Zarhopalus inquisitor (How.) (Encyrtidae; Hymenoptera)[2]

Pseudophycus meritorius Gahan (Encyrtidae; Hymenoptera)

Both of these parasites emerge about the time of oviposition by the overwintering forms. It is of interest to note that one female collected from its natural host produced 14 nymphs before the emergence of one *Z. inquisitor*. Ten *P. meritorious* Gahan have been noted to emerge from a single host; no more than two, and usually one *Z. inquisitor* emerged from a single individual of *F. virgata*. The following parasites, all of the Family Encyrtidae (Hymenoptera), have been recorded by the respective individual whose name appears opposite each:

Aenasius ianthinus Compere	Compere (1937)
Homalotylus mundus Gahan	Dammerman (1929)
Anagyrus coccidvorus Dozier	Dozier (1932)
Leptomastidea antillicola Dozier	Dozier (1937)
Holanusomyia Pulchripennis Girault	Otaner (1935)
Leptomastix longipennis Mercet	Otaner (1935)
Acerophagus texanus (Howard)	Timberlake (1916)
Blepyrus insularis (Cameron)	Timberlake (1921)
Pseudaphycus meracus Gahan	Gahan (1946)
Pseudaphycus sp.	Wolcott (1948)
Aenasius advena Compere	Zimmerman (1948)
Protaenasius sp.	Zimmerman (1948)
Leptomastidea abnormis (Girault)	Zimmerman (1948)

CHEMICAL CONTROL.—In August 1954, half of the plants under observation were treated with malathion at the rate of 1.5 quarts of 57% emulsifiable concentrate per 100 gallons. The plants were sprayed to the run-off point with a compressed-air hand sprayer. Subsequent observations failed to reveal any living mealybugs, despite the heavy infestation previously present. These plants remained almost completely free of infestation during 1955.

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Rearing Successive Generations of the Carpet Beetle under Controlled Conditions[1]

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The carpet beetles (Dermestidae: Coleoptera) are serious pests of wool, fur, feathers, silk, and many cereal grains and plant products. Consequently these pests are prevalent in warehouses, textile mills, furrier establishments, clothing stores, pillow and mattress factories, millinery shops, grain elevators, granaries, flour mills, feed stores, grocery stores, museums, and residences unless definite preventive measures are used to control and inhibit development of the pests.

The carpet beetle, *Anthrenus scrophulariae* (L.), is one of the four widely distributed pest carpet beetles in this country. The other three common species are the black carpet beetle, *Attagenus piceus* (Oliv.), the varied carpet beetle, *Anthrenus verbasci* (L.), and the furniture carpet beetle, *Anthrenus flavipes* (Lec.) All of these species are widely distributed, but apparently only

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three of the pests can maintain themselves continuously in the above environments. *A. scrophulariae*, during the adult stage, appears to require a period of freedom in outdoor environments. All four species can commonly be collected on many flowers, particularly Compositae and Scrophulariaceae, during the spring and summer months. Since the beetles so commonly feed on flowers, it suggests that some nutritive material obtained from the flowers is essential (Kunicke 1941). While only the carpet beetle appears to require this outdoor freedom to maintain itself (Greenwald 1941), some have had difficulty in rearing *A. verbasci* continuously under laboratory conditions (Yamada & Taniguchi 1940, Griswold 1941). This same factor from flowers does not seem to be required by the black and furniture carpet beetles, since they also are attracted to the same flowers, but can dispense with such feeding (Griswold 1941, Kunicke 1941).

All of the carpet beetles have been reared frequently in the laboratory (Takahashi & Uchiumi 1934; Back & Cotton 1936, 1938; Griswold 1941; Kunicke 1941; Fletcher & Kenaga 1942; Heal 1942; Moore & Moore 1942; Weinman 1942; Moore 1946; Haydak 1947; Zinkernagel 1951; CSMA 1952). However, the carpet beetle has not been reared continuously by any workers, but only after collecting the adults from outdoor environments where they apparently had access to certain essential foods not present in the usual laboratory diets (Greenwald 1941, Kunicke 1941).

Adult beetles of *A. scrophulariae* have been collected repeatedly from flowers and have reproduced in the laboratory. A high percentage of the offspring readily complete development under such conditions. These laboratory-reared adults, however, have not reproduced in the laboratory when kept under conditions suitable for reproduction and development of the other pest species of carpet beetles. A number of foods, including several species of greenhouse flowers and dandelions, have been offered to the beetles during the winter months. Feeding on a few of these flowers, particularly dandelion, occurred but without stimulating reproduction.

A number of laboratory-reared beetles were divided into two lots and offered four food ingredients, separately and in admixture: brewer's yeast, soybean flour, honey, and pollen. The pollen had been collected and stored by bees and may have been from one or more species of plants. Wool fabric sprinkled with yeast was added as an oviposition stimulus and larval food medium. Reproduction occurred in one of the cultures and soon a number of larvae were observed on the fabric. Additional fabric and powdered yeast were added as needed by the larvae. A thriving culture soon developed, but the number of larvae was not ascertained due to the pressure of other duties. The culture was not observed during an absence from February to September, during which time in spite of a food shortage a number of adults developed, a few of which were alive in September. Time did not permit attempting to continue the culture.

CONCLUSIONS.—A thriving culture of the carpet beetle, *Anthrenus scrophulariae*, was developed from laboratory-reared beetles. This is significant, not having been reported previously. The successful culture was offered a food admixture composed of brewer's yeast, soybean flour, and honey and pollen collected by bees. These findings do not necessarily solve the complete problem of inducing reproduction by laboratory-reared beetles.

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Influence of Nitrogen Fertilizer, Spacing, and Irrigation on Control of the Corn Earworm in Sweet Corn[1]

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Research by many workers has shown that DDT and DDT-mineral oil emulsion sprays are effective in control of the corn earworm, *Heliothis zea* (Boddie), in sweet corn. Almost without exception commercial growers of sweet corn in Alabama apply sprays for earworm control. The best growers at times obtain as much as 90 per cent worm-free ears; however, 60 to 75 per cent worm-free ears is probably nearer the average.

In an attempt to improve earworm control, various factors that may influence earworm infestations and control are being investigated in Alabama. An experiment was conducted in 1955 on the influence of irrigation, different rates of nitrogen fertilizer, and spacing of plants on the infestation of corn earworm in sweet corn that had been sprayed according to current recommendations.

It has been shown by Douglas & Eckhardt (1953) that under certain conditions the rate of nitrogen fertilization in corn did influence the amount of damage by the earworm. When the nitrogen deficiency was great enough to cause very low yields and small, poorly developed ears, earworm damage was significantly increased over plots receiving more nitrogen. If the amount of nitrogen was greatly in excess of the needs of the plant, more

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Table 1.—Earworm infestation following DDT emulsion sprays on sweet corn grown under varying conditions of irrigation, nitrogen fertilizer, and spacing of plants.

Nitrogen per Acre and Spacing of Plants	Per Cent Worm-Free Ears	
	Irrigated	Non-Irrigated
58 lb. nitrogen		
8-inch	62.5	59.5
12-inch	71.3	62.1
16-inch	62.5	71.0
88 lb. nitrogen		
8-inch	63.4	65.7
12-inch	65.9	63.3
16-inch	66.6	65.0
118 lb. nitrogen		
8-inch	64.8	70.2
12-inch	66.9	70.4
16-inch	68.0	72.5

earworm damage was found than that of corn receiving nitrogen in balance with the needs of the plant. An interesting situation relative to earworm in irrigated corn was found by Zuber (1955). He found that there was a greater penetration by earworms into the ears of non-irrigated corn than into ears of irrigated corn. Apparently there is little information on the influence of spacing of corn on the infestation by corn earworm in sweet corn ears. It has been shown by Eden (1950) that rice weevil, *Sitophilus oryza* (L.), damage in corn is inversely proportional to the number of corn plants per acre.

Procedure.—The study was conducted at the Gulf Coast Substation at Fairhope, Alabama. The variety of sweet corn was Golden Security. The experiment involved three rates of nitrogen and three spacings of sweet corn on irrigated and non-irrigated land. The plots consisted of 4 rows of corn 20 feet long. Treatments were replicated four times in randomized blocks. The weather was fairly seasonable and water was applied only one time to the irrigated plots, 1½ inches of water on June 6. The corn was sprayed seven times at 3-day intervals beginning on May 23, at which time approximately 25 per cent of the stalks were in silk. Two pounds of DDT in 15 gallons of emulsion spray was applied per acre per application with a high-clearance, self-propelled sprayer equipped with four flat fan nozzles per row. The spray was applied at 150 pounds pressure. All ears on the two inside rows of each plot were examined for earworms on June 14. A detailed statistical analysis was made of the data.

Results.—Results of the experiment are presented in table 1. The mean percentages of worm-free ears in the irrigated plot ranged from 62.5 to 71.3 with no significant differences between any of the treatments. The percentages in the non-irrigated corn ranged from 59.5 to 72.5 with no significant differences.

A complete and comprehensive statistical analysis revealed that in this experiment there were no significant effects on the percentages of worm-free ears due to irrigation, rate of nitrogen fertilizer, or spacing. Furthermore, the analysis revealed that the power of this experiment to detect significantly a difference of 6.6 percentage points was 0.8. Since such differences were not found, it was concluded with a probability of 0.8 that differences as large as 6.6 percentage points did not exist.

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A Caddy-Cart Type of Table for Grub Survey

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A collapsible caddy-cart table was built for use in sampling insecticide plots for grub populations and in making general white grub surveys. The golf bag attachments were removed and the cart equipped with a funnel, flexible steel hose, detachable table surface and a sampler carrying unit, figure 1.

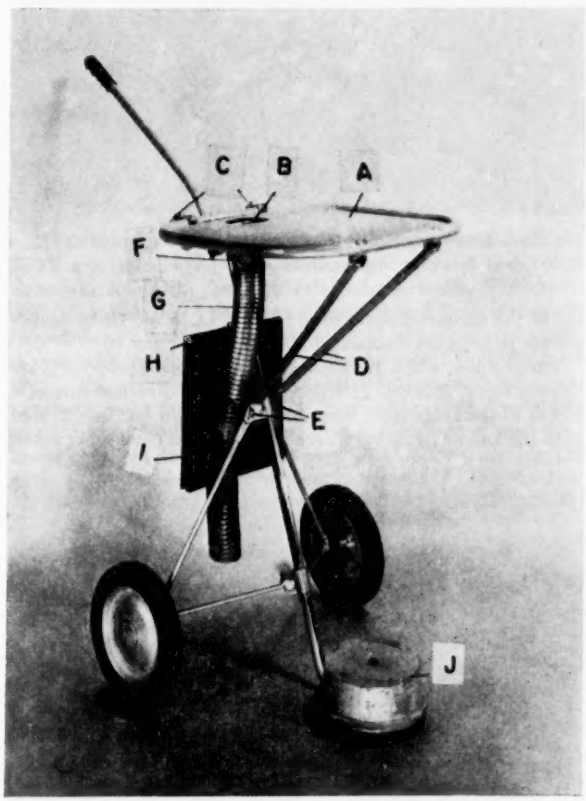

Fig. 1.—Shows caddy-cart table ready for use. Steel flexible hose replaced canvas hose in other figures.

The table surface is made of tempered wood fibre board (A) 18 by 22 inches with a hole (B) 3 inches in diameter cut in near one end. The board was mounted in an aluminum frame connected to the cart by two clamps that slip over prongs (C) attached to the cart and it is held in a level position by two braces (D). The upper ends of the braces are fastened to the table surface with a swinging joint and the lower slotted ends can be slipped over two bolts (E) without removing the winged nuts which when tightened hold the ends firmly. The hole (B) in the board is located directly above the funnel (F) and flexible hose (G) that are attached to the cart. The sampler carrying unit (J) is made of five pieces of ¼-inch plywood 7¼ inches in diameter and fastened firmly to the cart as shown in figure 1. The sampler or turf patcher is carried on this unit when the equipment is being moved from one place to another, figure 2. The cores of soil are transferred from the sampler to the table surface with the

FIG. 2.—Equipment being moved from one sampling area to another.

aid of the tempered wood fibre boards (I). When these boards are not in use, they are carried on hook (H).

The sampler, which is 8 inches in diameter, is used to procure the soil cores, figure 3. In order to get more than one core of 4 inches in thickness from the same hole, a cross bar was welded onto the handle 5½ inches above its base. As the cores are taken

FIG. 3.—Portable table in use. Also shows how soil samples are placed on small fibre boards before being transferred to table and how soil cores are broken apart for examination.

FIG. 4.—Equipment folded and stored in back compartment of a passenger car.

they are pushed out onto the boards (I) and then transferred to the table surface as needed. After the desired number of cores have been secured, the portable table is moved over the hole in the ground or turf with the bottom end of the flexible hose directly above the hole. As the soil cores are broken and the soil examined, the soil is pushed through hole (B) into funnel (F) where it falls through hose (G) back into the hole from which the soil was taken.

The complete unit can be folded and stored in the rear compartment of a passenger car for transportation from one sampling area to another, figure 4.

Further Tests with DDT and Pyrethrins-Piperonyl Butoxide Against the Hairy Spider Beetle[1]

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DDT and pyrethrins-piperonyl butoxide have been used extensively in Western Canada against the hairy spider beetle, *Ptinus villiger* (Reit.), a pest of warehouses in which cereal commodities are stored. The effectiveness of these insecticides against *P. villiger* was reported by Watters & Smallman (1953). During 1953, one company reported that DDT had failed to protect bagged cereal products from infestation. Experiments were therefore conducted to find out whether inadequate control with DDT was caused by misapplication. A similar experiment was carried out concurrently in warehouses of another company to determine whether pyrethrins-piperonyl butoxide was being applied effectively.

METHODS.—Rural warehouses were selected at random from lists of infested warehouses owned by the two companies. Nine of the warehouses selected for DDT treatment were treated by a laboratory officer; each of an additional 10 was treated by a different warehouseman of the first company. Ten of the warehouses selected for treatment with pyrethrins-piperonyl butoxide were treated by the laboratory officer; each of an additional eight was treated by a different warehouseman of the second company.

The laboratory officer used a knapsack pressure sprayer to apply the insecticides as a coarse wetting spray at 1 gallon per

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1000 sq. ft., the rate used previously (Watters & Smallman 1953). The floor and lower 5 ft. of the walls of each warehouse were sprayed in April and again in June. The warehousemen were instructed to apply the insecticides in a similar manner.

DDT[3] was supplied as a 25% (w:v) emulsifiable concentrate. This was diluted with water to give a 5% (w:v) emulsion. Pyrethrins-piperonyl butoxide[4] was supplied diluted with base oil to give 0.1% (w:v) pyrethrins and 0.8% (w:v) piperonyl butoxide. Good control with this concentration had been reported by several warehousemen during 1953 at an application rate of 1 gallon per 1000 sq. ft. although Watters & Smallman (1953) reported that at this rate a concentration of 0.25% (w:v) pyrethrins and 2.5% (w:v) piperonyl butoxide was required to give control comparable to that obtained with DDT.

P. villiger infests flour packed in cotton sacks when the female oviposits through the fabric. The number of progeny found in sacks indicates the extent of an infestation. Treatments were therefore assessed on the basis of counts of immature stages sifted from test sacklets of flour. Three 7-pound sacklets were placed on the floor of each warehouse after the April treatment (Smallman 1948). They were collected during September and sifted at the laboratory.

Analysis of variance was carried out with all data after an appropriate transformation, log $(x+1)$, was applied. Warehouses treated by the laboratory officer were regarded as replicates; all company-treated warehouses were considered as individual treatments.

RESULTS AND DISCUSSION.—The mean numbers of spider beetles found in three 7-pound sacks placed in each warehouse treated with DDT were as follows:

Laboratory-treated	5	2	6	5	0	0	0	3	4		
Mean	2.8										
Company-treated	0	3	2	26	0	0	136	0	306	47	
Mean	52.0										

Four out of ten company-treated warehouses had significantly higher numbers of spider beetles in test sacks than the mean of nine laboratory-treated warehouses. The remaining six warehousemen obtained control with DDT comparable to that obtained by laboratory personnel. The variation between company-treated warehouses was significantly greater than that between laboratory-treated warehouses. Reports of poor control with DDT against the hairy spider beetle during 1953 may be attributed to misapplication.

The pyrethrins-piperonyl butoxide formulation gave poor control of insects both in warehouses treated by the laboratory officer and in those treated by warehousemen. There was no significant difference in control between the two groups of warehouses. These results may be accounted for on the basis of the quantity of insecticide applied. At the applied dosage an average deposit of 4.5 mg. of pyrethrins and 36 mg. of piperonyl butoxide per sq. ft. would be obtained. This is less than the deposit of 10 mg. of pyrethrins and 100 mg. of piperonyl butoxide per sq. ft. found by Watters & Smallman (1953) to give residual control for about 6 weeks. The good control reported by warehousemen of one company during 1953 with a deposit of 4.5 mg. of pyrethrins and 36 mg. of piperonyl butoxide per sq. ft. applied every 6 weeks therefore appears questionable.

SUMMARY.—A 5% DDT emulsion applied by a laboratory officer at 1 gallon per 1000 sq. ft. gave good control of the hairy spider beetle, *P. villiger*, in warehouses. Six out of ten warehousemen also obtained good control. Reports of poor results with DDT during 1953 are attributed to misapplication.

Pyrethrins-piperonyl butoxide applied at a lower concentration than that usually recommended for residual treatment gave unsatisfactory control.

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[3] Green Cross 25% DDT emulsion, Green Cross Insecticides Ltd., Winnipeg, Manitoba.

[4] Ogilvie Flour Mills, Winnipeg, Manitoba.

Chlordane-Resistant Brown Dog Ticks and Their Control[1]

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Chlordane has been a recognized control for brown dog tick, *Rhipicephalus sanguineus* (Latr.), for several years but it failed to give control in a large dog kennel in New Jersey in the summer of 1955. At this particular kennel, the New Jersey Agricultural Experiment Station was asked to give assistance in developing a control program for a serious brown dog tick infestation during World War II when the only suitable control measure was the application of rotenone dust to the dogs.

When chlorinated hydrocarbon insecticides became available, DDT was tested and proved unsatisfactory, but in 1948 chlordane was found to give very satisfactory results. The brown dog tick infestation was practically eradicated by applying 4% chlordane as a residual spray to walls and ceilings of all kennels. A proposed preventive program called for monthly applications of 2% chlordane residual sprays and for 3 years, monthly applications were made. Then the interval between treatments was increased to 2 months and four applications a year were found sufficient to give control in 1953 and 1954. During the entire period, there were infrequent outbreaks of ticks of minor proportions and the owners suspected that additional ticks were being introduced from time to time.

In July 1955 the problem again became acute and residual treatments with chlordane were made. Three applications of 3% chlordane were made in a week in a portion of the kennel but the ticks remained uncontrolled. In addition some of the dogs were dipped in 2% chlordane both on the premises and by a veterinarian. In neither case, however, were ticks eliminated from the animals.

The Experiment Station was asked to help solve the problem and as a first step collected a sample of ticks for exposure to chlordane in the laboratory. For these tests, filter papers were first dipped in 2%, 5%, and 10% chlordane emulsions, respectively, and then, after drying overnight, were used to line large test tubes. Up to 20 live ticks were placed in a tube which was corked tightly and laid horizontally so that during the test period ticks were constantly exposed on the treated surfaces and to the vapors. Counts of live, moribund, and dead ticks were made daily. Ticks were recorded as alive if they could turn over and walk. Nymphs and unengorged males and females were exposed in one lot. Results of this test are shown in table 1. From these data it is evident that the ticks were able to withstand considerable exposure to chlordane. Even on 10% chlordane it took 5 days to kill all of the ticks by which time 75% of those in the untreated lot were dead or moribund. No provision was made for proper humidity or temperature and it can be assumed that conditions were not favorable to the ticks. The test seemed to show quite conclusively that ticks were chlordane-resistant even though no strain of ticks of known susceptibility was avail-

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[2] Associate Research Specialist in Entomology.

Table 1.—Constant laboratory exposure of nymphs and adults to chlordane residues on filter paper.

Exposure in Days	Tick Reaction to Exposure							
	2% Chlordane		5% Chlordane		10% Chlordane		Untreated	
	Moribund	Dead	Moribund	Dead	Moribund	Dead	Moribund	Dead
1	50%	5%	82.5%	2.5%	94.7%	0	40%	15%
2	75	5	65	27.5	89.4	10.6%	50	15
3	65	30	60	35	68.4	31.6	50	15
4	15	80	12.5	85	5.3	94.7	55	20
5	0	100	2.5	97.5	0	100	50	25
Ticks Exposed	20		40		19		20	

able for comparison. In the kennel, however, very large numbers of ticks had survived three applications of 3% chlordane.

TESTS WITH MALATHION.—Malathion was selected as the best and safest phosphate insecticide which might give control of these ticks and tests were instituted in which both regular and premium grades of the insecticide were used.

The kennel was so constructed that there were four similar wings with two rows of pens in each. The four wings were separated by a control room for food preparation, grooming, and similar activities. Each wing had tiled pens and the remainder of the walls and ceilings were covered with sheetrock wallboard. It was decided therefore, to use residual sprays of 1 and 2% premium grade malathion. At the same time, half of the dogs were treated in each kennel with 4% malathion dust where 1% residuals were applied, and with 1% malathion dips where 2% residuals were used. Infestations of the kennels were very heavy to moderate and dogs in all kennels were infested with 10 to 100 ticks.

Two other buildings, known as the isolation kennel and the processing kennel, were left untreated in the first tests. Both of these buildings had metal pens. Walls and ceilings were of painted concrete and thus presented a surface with abundant small cavities where seed ticks, nymphs, and unengorged adults were present in large numbers.

All treatments of dogs and all residual spraying except for the first wing sprayed, were made by employees of the kennel. Residuals were applied with a small estate-type sprayer operating at 100 p.s.i. and with a single nozzle gun. Fifty-seven per cent emulsifiable solutions were diluted to the proper strength for spraying. Dogs were removed from the kennels during spraying and until walls, ceilings, and floors had dried following spraying. Floors were hosed down after spraying to remove any excess material. The first wing was sprayed August 11 with 10 gallons of 1% malathion. On August 12, the second and third wings were sprayed and half of the dogs were treated in wings 1, 2, and 3. Treatments were made in wing 4 on August 15, and at that time the processing kennel was also sprayed with 2% malathion (premium) and all dogs were dipped in 2% malathion (premium).

Immediately after residual spraying, large numbers of ticks came out of protected places, revealing that the infestation was probably 10 times as heavy as was previously estimated. After spraying, samples of ticks were collected and taken back to the laboratory for observation. Most of these ticks had been wet thoroughly by the spray, though some were left in cracks and crevices and received their exposure mostly by walking on the material.

Mortality of ticks collected after spraying one wing with 1% malathion is shown in table 2. It is evident that this exposure was not sufficient to give control. Even a week after treatment more than 30% survived under unfavorable laboratory conditions. Kills in the kennel were somewhat higher because of longer exposure. Further treatments and observations showed that both 1% and 2% malathion residual sprays failed to give tick control though the 2% concentration was considerably superior to 1%. No difference was apparent between regular and premium grade material. Engorged females particularly seemed unaffected by the spray. Ticks which were alive or moribund were collected from the walls and in two pens sprayed August 12 all ticks on the floor were swept up and brought to the laboratory.

On 2% malathion an estimated 75% of the ticks were eventually killed though the material killed slowly and 3 of the 8 female ticks survived and laid viable eggs. The live ticks remaining in the kennel were still a serious problem.

On dogs, 4% malathion dust gave very little kill and the 1% malathion dip gave only partial kills. Many live ticks remained on the animals, especially in the ears even though special care had been taken to treat the ears thoroughly. A second dip was

Table 2.—Mortality of ticks collected from kennel sprayed with 1 per cent malathion on August 11.

Days After Spraying	Number of Ticks			Per Cent Dead
	Alive	Moribund	Dead	
1	37	6	0	0
3	23	4	16	37.2
5	17	1	25	58.1
7	14	0	29	67.4

given to a portion of the animals but failed to kill all ticks. In no case were harmful effects noted on the animals with either residual sprays, dusts, or dips of malathion. The odor, even of the premium grade, was somewhat objectionable.

TESTS WITH LINDANE.—Since numerous ticks still remained in the kennels and on dogs, it was decided that malathion was not the answer. Lindane was then selected for study and treatments were made on August 18 and 19 with a residual treatment of 0.5% lindane plus 1% lindane dust on half the dogs in one kennel. In another kennel only a spray containing 0.25% residual lindane was used. With the 0.5% lindane, as a precaution dogs were removed from the kennel for 3 days after spraying.

Results were promising when the kennels were examined 3 days later. Residual sprays gave fast kill and nearly complete elimination of ticks. In the heavily infested area sprayed with 0.25% lindane, only two live ticks were found. Most of the ticks on animals were killed when a 0.045% dip was used. Lindane dust appeared no better than malathion dust and gave no control. After the initial success with 0.5% lindane, it was decided to continue residual applications and to dip all dogs in 0.045% lindane. By early September only an occasional tick was found and the infestation was practically eliminated. The difficulty of treating all areas where ticks may be resting and the hardiness of the brown dog tick makes control difficult. At the present time in this kennel, lindane has given control and is the best material

tested. Malathion would be a fairly promising substitute if lindane should later fail.

SUMMARY.—From 1948 to 1955, 2% chlordane residual sprays had been used successfully to control brown dog tick, *Rhipicephalus sanguineus*, in a large dog kennel in northern New Jersey, but in 1955 a large infestation developed which could not be controlled with chlordane. In the laboratory, these ticks were found to withstand exposure to 2 to 10% chlordane residues. Malathion used as a residual spray at 1 and 2% concentrations gave partial control of these ticks, as did 1% malathion dips of dogs. Dusting dogs with 4% malathion and 1% lindane failed to give control. Lindane gave tick control when applied in the building as a residual spray at 0.25 or 0.5% concentration or applied to dogs as a dip containing 0.045% lindane.

The Effect of Pyrethrum Activators on the Toxicity of Sabadilla to House Flies[1]

MURRAY S. BLUM[2] and C. W. KEARNS, *Department of Entomology, University of Illinois, Urbana*

The ability of pyrethrum synergists to increase the toxicity of natural and synthetic insecticides has been demonstrated by many workers in the past decade. Brannon (1947) found that piperonyl butoxide and piperonyl cyclonene increased the toxicity of rotenone to the Mexican bean beetle. Gersdorff & Mitlin (1952) reported that the toxicity of scabrin (n-isobutyl-2,6,8-dodecatrienamide) to house flies was increased several times when piperonyl butoxide was applied along with the insecticide. Reed & Filmer (1950) showed that the plant alkaloid, ryania, was activated by piperonyl butoxide or n-propyl isome against the European corn borer. Perry & Hoskins (1950) reported that the toxicity of DDT to house flies was increased by piperonyl cyclonene. Hopkins & Hoffman (1955) found that the DDT analogue, Dilan, was synergized by piperonyl butoxide. Hoffman *et al.* (1954) demonstrated the activating effect of pyrethrum synergists on a series of oxygen substituted thiophosphates.

In connection with studies on pyrethrum synergists, the ability of pyrethrum synergists to increase the toxicity of the plant alkaloid, sabadilla, was determined.

MATERIALS AND METHOD.—Adult female CSMA house flies 3 to 4 days old were used in all studies. The flies were maintained on evaporated milk up to the time of treatment. During the observation period following treatment, the flies were fed a 2% solution of sucrose.

Acetone solutions of sabadilla and of the chemically pure synergists were applied to the CO_2-anaesthetized flies by means of a syringe-micrometer assembly. The synergists tested were piperonyl butoxide, piperonyl cyclonene, sulfoxide, MGK-264, I. N. 930, and n-propyl isome. In all cases, the ratio of synergist to sabadilla was 10 to 1.

Immediately after the treatment of three replicates of 25 flies in duplicate with the synergist, the sabadilla was applied. After treatment, the anaesthetized flies were placed in pint Mason jars, and mortality counts were made 24 hours later.

The data were submitted to a two-way classification analysis of variance (F-test) using a 95% level of significance. There was a significant difference between treatments. All treatments were found to be significantly different from the control by using Duncan's technique by comparison (Multiple range and multiple F-tests) (Duncan 1955).

RESULTS.—This investigation revealed that the toxicity of sabadilla towards the house fly is increased by the six pyrethrum synergists. Sulfoxide and piperonyl butoxide, two outstanding pyrethrum synergists, were the most effective sabadilla synergists.

Table 1.—The 24 hour mortalities of house flies topically treated with sabadilla and six pyrethrum synergists.[a]

SYNERGIST	μG. OF SYNERGIST	μG. OF SABADILLA	PER CENT KILL
Piperonyl butoxide[b]	5	0.5	50
	10	1.0	77
Piperonyl cyclonene[c]	5	0.5	32
	10	1.0	56
Sulfoxide[d]	5	0.5	48
	10	1.0	72
MGK 264[e]	5	0.5	14
	10	1.0	53
I. N. 930[f]	5	0.5	19
	10	1.0	52
n-propyl isome[g]	5	0.5	38
	10	1.0	52
Sabadilla	—	0.5	8
	—	1.0	19

[a] Mortalities with the 10 μg. of each synergist were 5 ± 2% including an additional treatment with acetone.
[b] 3,4-methylenedioxy-6-propylbenzyl (butyl) diethyleneglycol ether.
[c] Condensation product of the alkyl-3,4-methylenedioxystyryl ketones with ethyl acetoacetate.
[d] 1-methyl-2-(3,4-methylenedioxyphenyl)-ethyl-n-octyl-sulfoxide.
[e] N-(2-ethyl hexyl) bicyclo (2,2,1)-5-heptane-2,3-dicarboximide.
[f] Isobutyl-10-undecylenamide.
[g] Dipropyl ester of naphtho (2,3)-1,3-dioxole-5,6,7,8-tetrahydro-7-methyl-5,6-dicarboxylic acid.

[1] Investigation supported in part by Research & Development Division, Office of the Surgeon General, Department of the Army, under Contract #DA-49-007-MD-306. Accepted for publication November 1, 1955.
[2] Present address: Quartermaster Research & Development Command, Natick, Mass.

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EDITORIAL

Our Society as Seen from a Distance

Before World War II, when he was an entomologist in fact as well as by adoption, the writer was among those who dreamed of a single strong national entomological society with subordinate branches and sections—a society that would stand high among the biological societies of the United States, representing both the biological and economic aspects of entomology, cooperating with other organizations in the larger interests of biology and of science in general, and taking advantage of every opportunity to make entomology and entomologists most useful. Since the war the organization that once seemed unattainable has been achieved by the devoted efforts of many entomologists. As an interested person who did not take part in the travail of the reorganization, the writer would like to make some observations on the correspondence between his earlier hopes and present reality.

The new Entomological Society of America, if given adequate funds, is now in a position to do everything previously envisaged. It has its own office with competent staff, an advantage enjoyed by few biological societies. It has an excellent organizational structure, responsive to geographical and subject interests of the members of the Society. As its procedures are thoroughly democratic, its progress depends on the course that its members wish to follow. The writer would like to persuade the membership that the Society should consciously avoid the danger of becoming an isolated trade union of economic entomologists, a real danger arising from democratic procedures in the nomination and election of officers and from the simple fact that economic entomologists outnumber biological entomologists.

When the new Entomological Society of America was formed, Charles E. Palm was to have been the next president of the American Association of Economic Entomologists and Herbert H. Ross the next president of the old ESA. Dr. Palm became president of the new ESA and, very properly, Dr. Ross succeeded him. However, no provision for that kind of economic-biological alternation was made for the future with the result that three economic entomologists follow Dr. Ross, and unless some scheme of rotation is adopted for the periodic election of a biological entomologist, economic entomology will dominate, or appear to dominate, the Society.

Why, it may be asked, should anyone be alarmed by the prospect of domination of the Society by economic entomologists? Is not the existence of entomology as a branch of science apart from zoology dependent entirely upon its economic aspects? Yes, but economic entomology is not just entomology; it is a heterogeneous mixture of entomology, business, economics, education, law, medicine, agriculture, engineering, physics, chemistry, biology, and statistics, all applied in various proportions and combinations to the control of insect pests. Economic entomologists are rarely outstanding in any one of the components of their profession. They are responsible for great advances in human welfare but are seldom recognized as eminent scholars, because they cannot concentrate upon any of the recognized subdivisions of human knowledge. So it is that scholars in other scientific organizations are inclined to overlook economic entomologists. Therein lies the danger of permitting the new ESA to appear to be dominated by economic entomologists. The greater the identification of the Society with economic entomology, the fewer may be its contacts with the more scholarly societies, and the lower its prestige may fall among leaders of American science.

If the above analysis is correct, nominations for the office of President-elect ought to be controlled so that at regular intervals a biological entomologist will be elected. In arranging a system of rotation, it would be well to provide also for alternation between official and commercial economic entomologists, as has been customary in the Eastern Branch of ESA. Participation by commercial entomologists in the affairs of ESA is just beginning and should be encouraged by periodic selections or elections from the ranks of these experienced and very able men.

Another excellent preventive of isolation of ESA is active participation of the Society in the affairs of the American Association for the Advancement of Science and the National Academy of Sciences—National Research Council. This depends in part on the interests and initiative of the Society's representatives in these over-all scientific organizations and in part on the opportunities that are given them for participation. Much can be done by them to advance the recognition of entomology.

Above all the Society should become a member of the American Institute of Biological Sciences as soon as possible. As altruistic arguments for such membership have had no effect, let us consider what active membership in AIBS could do for the Society. As one of the larger biological societies, ESA could exert considerable influence on the development of the newly independent AIBS, causing it to plan and undertake activities of benefit to the Society. For example, economies in publication that are possible through joint action of the societies in AIBS may be arranged for the distinct benefit of ESA. Is it not better for ESA to take part in this development, which will surely come, than to remain on the outside without a voice in the procedures and services that will be set up? Even if no financial profit from membership in AIBS can be demonstrated for some years, is it not wise for ESA to get the intangible benefits of working together with other societies for the common good? Here is a way to get esteem, which entomologists value as much as money; and here is a way to get money, too, for AIBS is closer to the fund-granting agencies than are the individual societies.

The writer feels so strongly that ESA should now become a full member of AIBS, that it is hard for him to refrain from belaboring the issue. Looking at ESA from a distance, he sees his beloved Society suffering from tendencies toward isolationism. In view of the magnificent progress made by the Society since the war, he is confident that in due time ESA will play a leading role in the community of biological societies.

FRANK L. CAMPBELL,
National Research Council,
Washington, D. C.

OBITUARY

Edgar William Davis

1895-1954

Mr. Edgar William Davis died suddenly from a heart attack at his home in Union Gap, Washington, on October 24, 1954. He had not been in good health for several years, but his sudden death was a shock to his family and friends. He is survived by his wife, Lola, and daughter, Dixie.

Mr. Davis was born in Lyons, Kansas, on March 18, 1895 and spent his youth and early manhood in this area. At the outbreak of World War I, he entered the army and later saw combat and occupational service in France. While in combat he incurred an injury which affected his hearing in one ear. Following his discharge from the Service, Mr. Davis entered Kansas State College and subsequently received his B.S. and M.S. degrees in entomology at this institution. He was a member of the Ancient Free and Accepted Masons and American Association of Economic Entomologists.

Mr. Davis joined the Division of Truck Crop and Garden Insect Investigations of the Bureau of Entomology, now the Entomology Research Branch, early in 1925. His first assignment was that of field assistant on onion maggot studies in Madison, Wisconsin. After several months he was transferred to Toppenish, Washington, to initiate studies on the biology and ecology of the beet leafhopper. Subsequently, Mr. Davis was transferred to Richfield, Utah, to start a new laboratory devoted to the study of the beet leafhopper. This laboratory was moved to Salt Lake City, Utah, in 1930. In 1936, Mr. Davis' headquarters were moved to St. George, Utah, to initiate studies on control of pests of sugar beet seed production in that area. He was later shifted to Modesto, California, to conduct studies on beet leafhopper control in tomatoes. In 1943, he was transferred to Union Gap, Washington, where he conducted research on potato insects until his death.

In the course of his 30 years as an entomologist, Mr. Davis made many worthwhile contributions to our knowledge of the ecology of the beet leafhopper. His work on the overwintering and migration habits of this insect in the intermountain region deserves special mention. Later, he made equally outstanding contributions on the biology and control of potato insects, especially aphids. He possessed an unusually good collection of leafhoppers of Utah and prepared a rather comprehensive check list and catalogue of the leafhoppers of the United States. Mr. Davis is author of a number of scientific articles.

Mr. Davis was a very enthusiastic and energetic man, always eager to get a job done and prided himself in completing an assignment with credit to the organization with which he was associated.

William C. McDuffie
Howard E. Dorst

Edgar William Davis

BOOK REVIEWS

MOSQUITOES OF NORTH AMERICA (NORTH OF MEXICO), by Stanley J. Carpenter and Walter J. LaCasse. University of California Press, Berkeley and Los Angeles. 1955. 11¼×9 inches, 360 pages, 288 figs., 127 plates. $10.00

This large volume contains 127 full page splendid drawings of female mosquitoes rendered by Japanese artists of the 406th Army Medical General Laboratory. The 288 text figures are excellent line drawings of the male genitalia and the characteristics of the 4th instar larval parts useful in identification. Many of the text figures were also prepared by the Japanese artists. A considerable number of the figures were borrowed from the 1946 publication on the mosquitoes of the Southern States by Carpenter, Middlekauff and Chamberlain, and the remainder were done by Eustorgio Mendez and Reginald Jones. The localities in which the illustrated specimens were collected are not given.

The publication contains short chapters of two and three pages each on Life History, Collecting, Preparing Specimens for Study, and Internal Anatomy of the Female Mosquito, and a longer chapter (15 pages) on External Anatomy. The rest of the book, from page 25, contains keys, detailed descriptions of adults and larvae, distribution by states and provinces, and brief summary statements on bionomics and relation to disease. The pupae are not described. A Bibliography of 775 titles and a Systematic Index are included. It is stated on the jacket that 143 species and subspecies belonging to 11 genera are described. The jacket also contains the statement that this is a definitive work on taxonomy, biology, geographical distribution and medical importance but the term is hardly applicable to the very brief statements on bionomics and medical importance since one must go to other sources for most of the details. The descriptions and drawings appear to have been very carefully prepared. However, my attention has been called to an error in the key to *Wyeomyia* females where *haynei* and *smithii* are placed in the couplet with entirely dark middle and hind tarsi whereas the text correctly refers to white markings on the mid tarsi in both species. The described markings of the hind tarsi and the anterior pronotal lobes of *hayeni* should also be clarified as they differ from those given by Yamaguti and LaCasse, as do also some of the described markings of *vanduzeei*. This well printed and well bound book will be of particular value to mosquito taxonomists and to anyone who has occasion to identify North American species. The other information included, however brief, and especially the handsome full-page plates of female mosquitoes should have even a wider appeal.

W. V. KING

THE BLACKFLIES (DIPTERA, SIMULIIDAE) OF GUATEMALA AND THEIR ROLE AS VECTORS OF ONCOCERCIASIS, by Herbert T. Dalmat, 1955. Smithsonian Miscellaneous Collections, Volume 125, Number 1, Washington, D.C.; 425 pages, 44 plates, 21 figures, 36 tables, and 15 maps. Price $5.00.

Although the literature on black flies is voluminous, there are few works on these insects that are truly monographic in nature. Dalmat's publication on the black flies of Guatemala is not only one of the largest, but it is the most complete geographical monograph on the Simuliidae. From the detailed table of contents to the unusually complete index, the entire 425 pages are packed with useful and clearly presented information. The cost of publication was financed by the Smithsonian Institution and the Pan American Sanitary Bureau.

The importance of these flies both as pests and as disease vectors is discussed. A large section is devoted to the epidemiology of the disease known as Onchocerciasis, caused by the nematode, *Onchocerca*, that is transmitted to man by the black flies. About half the text includes the taxonomy of the 41 species found in that country. The techniques of collecting and handling the study material is given in some detail. It is a pleasure to find in the taxonomy section a good glossary of the terms used and also illustrations to depict most of the important characters in the keys. Natural keys separate both sexes of the adults, the larvae and the pupae. The generic nomenclature follows the more conservative views and the three genera *Cnephia*, *Gigantodax* and *Simulium* are sub-divided into sub-genera. Dalmat has included descriptions of each species in minute detail and a number of life stages and adult forms are described for the first time which adds to the usefulness of this work. It is not often possible to publish complete descriptions of each species and it is encouraging to find all the detail in this one publication. However, the many pages of detailed descriptions seem somewhat unnecessary as most of the forms and all of the species are adequately described elsewhere. For most workers, it would have been sufficient to describe only the diagnostic characters except for all new forms or stages.

In the section on distribution, species are listed in two ways. First, a species list including the various departments and localities where the species occur. Next is a department or locality list with the species that occur in each department.

The life history and biological studies include the feeding habits of the adults, especially for the three anthropophilic species *S. ochraceum*, *S. metallicum* and *S. callidum*. Ecology is given a separate subject heading and is particularly complete for the immature stages. The habits of the adults seem well correlated with the disease transmissions and special attention has been given to the anthropophilic species.

The number of the references cited is large, but rather modest for a work of this type and a number of references on the biology-ecology of black flies might have been included.

There are three sections in the appendix that show plant associations and the fauna of a portion of the study area. In addition, there are a number of charts and graphs that give much detail concerning the life history and ecology of the anthropophilic species, the associated species, feeding and resting habits of the adults and the distribution of the species according to altitude.

The drawings are generally of excellent quality; however, a few are somewhat over-simplified. The publishers are to be commended for including so many interesting as well as informative half tones and special mention is made of the color plate.

B. V. TRAVIS
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THE MEGACHILINE BEES OF CALIFORNIA (HYMENOPTERA: MEGACHILIDAE). P. D. Hurd, and C. D. Michener. 1955. Bull. Calif. Insect Survey 3: 1–247. Univ. of California Press, Berkeley. $3.50.

If you expect a full course meal with *Megachile* and *Osmia* as the *pieces de resistance*, you may be disappointed to find only a cold plate special composed of the smaller megachiline genera. However, you needn't go hungry; the servings are adequate and carefully prepared.

Native sons will swell with pride to learn that of the 154 North American species in the eight genera treated, 116 are known from California. The authors give complete distributions and host records for all Californian species as well as those suspected of occurring within the State boundaries. It seems a shame that a treatment so close to being a continental revision wasn't made one.

The introduction summarizes knowledge on the bionomics of the subfamily and briefly discusses floral relationships and distribution. Pollination is discussed along with a stirring appeal for the introduction of bee species specifically adapted to some

of our introduced crops. Although the authors discuss all of the megachiline genera in the introduction, they fail to mention *Megachile* and *Osmia* as pollinators of economic crops. These genera happen to contain the most important pollinators in the subfamily.

In the systematic section, which comprises the bulk of the book, the authors present a full discussion of the distributional pattern, taxonomic relationships, floral ecology, and the few known facts concerning the biology of each genus. They treat the species in a similar but briefer manner.

The routine taxonomic treatment contains keys to the genera, subgenera, species, and subspecies found in California and neighboring areas. The genera and subgenera are briefly characterized but the species are described only when the authors seem to feel the keys need fortifying. Locality, season, host, collector, and repository data are fully given for specimens collected in California but in most cases only summarized for other parts of the continent. Distribution maps for every species comprise an outstanding feature of the book. Locality records are spotted on the maps and estimated ranges are shaded. Each genus is illustrated by a sketch of the lateral aspect of a representative species. Simple line drawings illustrate diagnostic features of most of the species.

This volume is the most ambitious of the insect survey series yet to appear. It gives us hope that some of us will live long enough to see the day when order instead of chaos will be the rule in our knowledge of the western insect fauna.

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U. S. Department of Agriculture

Control of the Raspberry Root Borer[1]

LEW E. WALLACE,[2]
Oregon State College, Corvallis

The raspberry root borer, *Bembecia marginata* (Harr.), family Aegeriidae, is an important pest of cultivated cane berries in Oregon. The larvae feed on the roots and crown of their host for 2 years before transforming to the pupal stage. Just before pupation an emergence tunnel is made in the base of an infested cane. A thin covering of epidermis remains over the future emergence hole of the adult. The feeding of the larvae on the roots and crowns weakens the plants and the emergence tunnel kills the individual canes. At Milton-Freewater, Oregon, tests to control the adults or larvae were made in 1952 and 1953. Examinations of the plants to measure the amount of control were made in 1954 and 1955, 2 years after the applications were made for control.

Each test plot consisted of three rows of red raspberries 60 feet long. Each treatment was replicated four times at random. Treatments were applied September 6, 1952, and September 11, 1953. These dates were 18 and 21 days, respectively, after adult emergence had begun. The sprayer was operated at 300 pounds pressure. The amount of spray used was 1,512 gallons per acre in 1952 and 756 gallons in 1953. Spray was directed at the lower 2 feet of the plants which caused some puddling around the crowns. The soil in the test field was rocky and the spray readily seeped in around the crowns of the plants. The weights of the diluted wettable powders used per 100 gallons of spray are listed in table 1.

Table 1.—Summary of raspberry root borer control plot results.

Treatment	Pounds /100 Gallons	1954 Totals			1955 Totals		
		Larvae	Pupae	Per Cent Control[a]	Larvae	Pupae	Per Cent Control[a]
DDT 50%	4	0	0	100	2	1	86
DDT 50%	8	1	1	82	1	2	86
Parathion 25%	4	0	0	100	0	0	100
Parathion 25%	8	0	0	100	0	0	100
Chlordane 40%	4	4	4	27	6	5	50
Chlordane 40%	8	16	11	—	8	7	32
Untreated		9	2	—	19	3	—

[a] Control was based on larvae plus pupae in the untreated plots.

Five plants from the center row of each plot were dug between August 5 to 11 in 1954 and August 22 to 29 in 1955. These plants were carefully examined for root borer larvae and pupae. Results of the number of larvae and pupae found in the four plots for each treatment are shown in table 1.

Chlordane gave no control at the 8-pound level in 1954 and poor control at both levels in 1955. DDT showed considerable control at both levels in 1954 and 1955. Parathion at both levels gave excellent control in both years.

[1] Accepted for publication January 18, 1956.

[2] Formerly Assistant Entomologist at the Milton-Freewater Experimental Area; now of Entomology Research Branch, Agr. Res. Serv., U.S.D.A., Bozeman, Montana. Mr. Earl Brown began the work in 1952. The author acknowledges the aid of Dr. R. G. Rosenstiel of Oregon State College, who gave advice on the problem and helped edit this paper.

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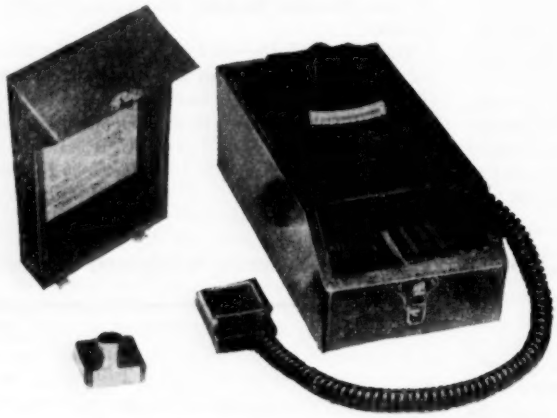

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